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**THE SANDFLY VECTORS AND EPIDEMIOLOGY OF CUTANEOUS
LEISHMANIASIS IN THE LANDAZURY FOCUS, COLOMBIA**

**Thesis submitted for the degree of Doctor of Philosophy in the
University of London**

**by
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ABSTRACT

This thesis describes a comprehensive cohort study of cutaneous leishmaniasis (CL) in a population of 2,704 followed over a period of 19 months, in the Opon focus, Landazury, Santander, Colombia. **Chapter 1** reviews the public health importance of CL at international, national (Colombia), and local (Santander) levels. Particular attention is given to the distribution of *Leishmania* parasites, sandfly vectors and reservoirs in Central and South America, according to the ecological regions defined by the Andes mountain range. **Chapter 2** describes the materials and methods of the whole project, emphasising its clinical, epidemiological and entomological aspects, respectively. Each one of the following three chapters contains an introduction, results and discussion. **Chapter 3** addresses the diagnosis, aetiology and clinical symptoms of leishmaniasis patients. The principal findings were as follows: (i) from three methods tested, PCR and the direct examination of slides are recommended as diagnostic tools in distant leishmaniasis foci; (ii) the main parasite circulating in Opon is *L. panamensis*; (iii) cutaneous lesions were larger if patients were infected at a younger age, and tended to be located on the face of children, on the legs of women, and on the torso of man; and (iv) about 10.2% of CL patients had mucosal lesions of low severity. **Chapter 4** presents the results of the cross-sectional survey carried out between May-July 1995, and the results of the 19 months prospective survey.

Results are divided into sections on the population structure; the population transmission rate for infection and disease; the personal, household and village risk factors for infection; seasonal variation in the incidence of leishmaniasis; the risk factors for developing clinical symptoms with infection; acquired immunity; and finally an analysis of the potential bias in the study. The main findings were as follows: (i) the cumulative prevalence of infection amongst the whole study population was 0.75; (ii) the average transmission rate in this focus is currently ca. 0.19/year; (iii) the risk of infection is equal for both genders and for all ages; and (iv) transmission was less likely in houses surrounded by secondary forest. In **Chapter 5** the sandfly fauna in the focus are described, focusing on their seasonal and nocturnal activity patterns; the relationship between habitat type and indoor sandfly abundance; the spatial relationship between abundance and transmission rate; and the natural infection rate with *Leishmania*. The main findings were as follows : (i) the principal vectors in the Opon focus are *Lu. trapidoi* and *Lu. gomezi*; (ii) a significant proportion of transmission to humans takes place indoors and at night; and (iii) the widespread deforestation that characterises the Opon focus has not caused any reduction in the incidence of leishmaniasis, presumably because the sandfly vectors continue to breed successfully in the cacao plantations that have replaced much of the primary forest. **Chapter 6** provides a discussion of the complete project, focusing on the significance for public health in Santander.

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My training in sandfly identification and the supply of some indispensable reagents to the project were received from the National Health Institute of Colombia, and I want to thank Dr Cristina Ferro and Dr Santiago Nicholls for their advice and support. Some biological samples were processed in the

Molteno lab in Cambridge University, where Dr. Douglas Barker, Sharon McCann and Susan Brewster generously helped me. Other samples were processed at the London School of Hygiene and Tropical Medicine with the help of Debbie Nolder who shared with me her expertise. Many people from the London School of Hygiene and Tropical Medicine helped me on different times of my work, especially, Rupert Quinnell, Paul Coleman, Orin Courtenay, Mary Cameron, Cheryl Cooper, Patricia Escobar, and Raul Pardo.

All the people mentioned above not only worked with me, I also was lucky that every one became my friend. This work was generously sponsored by COLCIENCIAS, Colombia. Finally, I want to dedicate this work to my wife Betsi Rueda Carvajal.

STATEMENT OF RESPONSIBILITY FOR THE STUDY

Field survey. Gerardo Munoz-Mantilla (GMM), carried out the complete census i.e. filling the census sheets, giving the MST and reading the MST result. The former malaria control program team assisted by painting the codes on each house and by mapping the villages. **Patient diagnosis and treatment in the field.** GMM collected most of the samples for parasite diagnosis (giemsa, culture and PCR) with the assistance of Reynaldo Gutierrez. Patients were treated and followed-up by the physician Jairo Pinzon. **Diagnosis studies in the laboratory.** The giemsa-stained slides were mostly observed by GMM, with the assistance of Reynaldo Gutierrez. Cultures were maintained and cryopreserved in Bucaramanga by Gloria Casadiego and Elsa Morales. Isolates were characterised either by GMM using isoenzymes in LSHTM (following training by D. Nolder) or by Elsa Morales, using monoclonal antibodies in the National Health Institute of Bogota (INS) (following training by Santiago Nichols and Sofia Duque) by monoclonal antibodies. GMM also carried out all the PCR diagnosis in Cambridge University (followed training by Sharon McCann). **Entomology.** Sandfly collections were made by GMM and a team of three field assistants. Sandfly identification was carried out by GMM, with the assistance of Cristina

Ferro and Magaly Sandoval, in Bucaramanga and Bogota (INS). Sandfly dissection for parasites was carried out by GMM in Bucaramanga with the assistance of Magaly Sandoval and Adriana Reyes. PCR on positive sandflies was carried out by GMM in Cambridge University. **Analysis.** Statistical analysis on all the data was carried out by GMM in LSHTM, following training by Clive Davies.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
STATEMENT OF RESPONSIBILITY FOR THE STUDY	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	xv
LIST OF FIGURES	xviii
ABBREVIATION LIST	xxii
1. INTRODUCTION	1
1.1 THE NATURAL HISTORY OF LEISHMANIASIS	1
1.2 LEISHMANIASIS AS A PUBLIC HEALTH PROBLEM IN SOUTH AMERICA	4

1.2.1 <i>Leishmania</i> species distribution in America	7
1.3 LEISHMANIASIS AS A PUBLIC HEALTH PROBLEM IN COLOMBIA	13
1.3.1 Introduction	13
1.3.2 The distribution of <i>Leishmania</i> species in Colombia	14
1.3.3 Regional variation in the annual Incidence of LCL in Colombia.	17
1.3.4 Variation in the annual incidence of LCL in Santander Department, according to Municipalities	18
1.4 RATIONALE AND OBJECTIVES OF THE OPON FOCUS STUDY	24
2. MATERIALS AND METHODS	29
2.1 STUDY AREA	29
2.2 STUDY DESIGN	39
2.2.1 Human population study	39
2.2.1.1 Cross-sectional Survey	39
2.2.1.2 Montenegro Skin Test (MST)	41
2.2.1.3 Prospective longitudinal study	42
2.2.1.4 Measurement of transmission rates	43
2.2.2 Sandfly Vectors	45
2.2.2.1 Collecting Methods	45
2.2.2.2 Species Identification	46
2.3 DIAGNOSTIC METHODS	47
2.4 PARASITE CHARACTERISATION : ISOENZYME ELECTROPHORESIS (IEA)	49
2.5 NATURAL INFECTION OF SANDFLIES	50
2.6 STATISTICAL ANALYSIS	51

2.6.1 Model simplification	51
2.6.2 Factor level simplification	52
2.6.3 Significance testing	52
2.7 ETHICAL CONSIDERATIONS	53
 3. DIAGNOSTIC AND CLINICAL FEATURES OF LEISHMANIASIS IN THE OPON FOCUS	 54
 3.1 INTRODUCTION	 54
 3.2 RESULTS	 56
3.2.1 Diagnostic methods	56
3.2.2 Parasite classification	60
3.2.3 Factors determining the size, number and site on the body of lesions or scars	62
3.2.3.1 Risk factors associated with the size of lesions or scars	62
a) Size of lesion	63
b) Size of scars	65
3.2.3.2 Risk factors associated with the number of lesions or scars	67
a) Number of LCL	68
b) Number of scars	68
3.2.3.3 Risk factors associated with the localisation on the body of lesions or scars	70
a) Localisation on the head	71
b) Localisation on the arms	73
c) Localisation on the legs	73
d) Localisation on the trunk	74
3.2.4 Comparison of patients in relation to the results of parasitological diagnosis and spontaneous healing rate	79
3.2.4.1 Risk factors for a positive parasitological diagnosis	80

3.2.4.2 Risk factors for a chronic lesion	83
3.2.5 Mucosal leishmaniasis (MCL)	84
3.3 DISCUSSION	86
3.3.1 DIAGNOSTIC METHODS	86
3.3.1.1 Microscope examination (ME)	87
3.3.1.2 Culture of parasites	89
3.3.1.3 Polymerase Chain Reaction (PCR)	90
3.3.2 PARASITE CLASSIFICATION	92
3.3.3 CLINICAL FEATURES	93
4. EPIDEMIOLOGY	96
4.1 INTRODUCTION	96
4.2 RESULTS	99
4.2.1 Population structure	99
4.2.2 Population infection rate	103
4.2.3 Population clinical infection rate	108
4.2.4 Personal risk factors for infection	110
4.2.4.1 Gender	110
4.2.4.2 Age	110
4.2.4.3 Clinical status.	113
4.2.5 Household risk factors for infection	114
4.2.6 Village risk factors for infection	117
4.2.7 Risk factors for clinical symptoms with infection.	123
4.2.8 Acquired immunity against disease	127
4.2.9 Recovery rate	132
4.2.10 Recurrent leishmaniasis	134

4.2.11 Seasonal transmission	135
4.2.12 Mucocutaneous leishmaniasis (MCL)	139
4.2.13 Identification of likely sources of bias	140
4.2.13.1 Potential bias through the selection of the “study population”	140
4.2.13.2 Potential bias through incomplete follow-up of the study population	142
a) Emigrants	142
b) Refusal group	143
4.3 DISCUSSION	145
4.3.1 Transmission rates	148
4.3.2 Risk factors for infection	150
4.3.2.1 Personal risk factors	150
4.3.2.2 Risk factors associated with land use	151
4.3.2.3 Seasonality	153
4.3.3 Risk factors for clinical/subclinical infection	154
4.3.3.1 Recurrent leishmaniasis	158
4.3.3.2 Acquired immunity	160
5. SANDFLY VECTORS	162
5.1 INTRODUCTION	162
5.2 RESULTS	166
5.2.1 Description of the sandfly fauna in the Opon focus	166
5.2.1.1 Man-landing collections	168
a) Seasonal variation study	168
b) Nocturnal activity study	170
c) Natural infection study	172
5.2.1.2 Shannon trap	173

5.2.1.3 CDC light traps	173
5.2.1.4 Resting places: tree trunks	176
5.2.1.5 Additional collections in the region	176
5.2.2 Natural infection of sandflies	177
5.2.3 Nocturnal activity	178
5.2.4 The effect of vegetation on variability in indoor sandfly abundance	184
5.2.4.1 A description of the sandfly database	184
5.2.4.2 The relationship between the sandfly fauna in a house and its surrounding vegetation	187
5.2.5 The relationship between indoor sandfly abundance and transmission rate.	189
5.2.5.1 Variation at the household level	189
a) Incidence	189
b) Prevalence	191
5.2.5.2 Variation at the village level	192
a) Incidence	192
b) Prevalence	194
5.2.6 Seasonal variation	195
5.3 DISCUSSION	201
5.3.1 Vectors of <i>Leishmania panamensis</i>	201
5.3.2 Natural infection of sandflies	203
5.3.3 Collection methods	207
5.3.4 Vector incrimination by regression analysis	209
5.3.5 Geographic distribution	211
5.3.6 Vegetation in relation to sandfly abundance	213
a) Cacao crops.	214
b) Pasture and secondary forest	216
c) Effects of habitat change on sandfly fauna: conclusions	217
5.3.7 Sandfly activity in the domestic environment	218

a) Peridomestic activity	218
b) Endophagic behaviour	219
c) Dispersal to the domestic environment from the surrounding habitat	220
5.3.8 Nocturnal activity of sandflies	222
a) <i>Lutzomyia trapidoi</i>	223
b) <i>Lutzomyia gomezi</i>	226
c) <i>Lu. hartmanni</i> , <i>Lu. yuilli</i> and <i>Lu. quasitowsendi</i>	228
5.3.9 Seasonal variation study	229
a) <i>Lu. trapidoi</i>	232
b) <i>Lu. gomezi</i> .	233
c) <i>Lu. hartmanni</i>	234
d) <i>Lu. quasitowsendi</i>	234
 6. CONCLUSIONS AND RECOMMENDATIONS	 237
 REFERENCES	 244
 APPENDIX	

LIST OF TABLES

	Page.
Table 1.1 Recognised Leishmania species causing human diseases in the New World	9
Table 1.2 Distribution of cutaneous leishmaniasis in Colombia by regions	20
Table 2.1 Climatic conditions in the Opon focus, 1996	38
Table 3.1 Distribution of leishmaniasis test results by method	59
Table 3.2 Concordance of diagnostic methods	59
Table 3.3 Sensitivity of diagnostic methods	59
Table 3.4 Measurement of selection bias in the application of diagnostic test	59
Table 3.5 Risk factors associated with area and number of active lesions (univariate analyses)	64
Table 3.6 Risk factors associated with area and number of scars (univariate analyses)	66
Table 3.7 Risk factors associated with the localisation on the body of lesions or scars (univariate analyses)	75
Table 3.8 Minimal Adequate Model for risk factors associated with area, number and localisation of scars and lesions	76
Table 3.9 Risk factors associated with spontaneous healing and parasitological positive diagnostic tests	82
Table 3.10 Minimal adequate models for predicting spontaneous healing and a positive parasitological diagnosis	83
Table 4.1 Distribution of the study and excluded populations by age, clinical status and MST	101
Table 4.2 Distribution of the study population by clinical, MST status, MST conversions and villages	102
Table 4.3 The relationship between clinical status and MST size	104

Table 4.4 Distribution of the study population by clinical and MST status including follow up	105
Table 4.5 Force of infection and Recovery rate calculated from MST age prevalence data	109
Table 4.6 Force of infection and Recovery rate calculated from clinical status age prevalence data	109
Table 4.7 The MST conversion rate by age	112
Table 4.8 The number of houses in each village with MST survey and vegetation coverage data	115
Table 4.9 Household and village risk factors for infection: Vegetation type.	116
Table 4.10 Minimal adequate model for risk factors of clinical infection, recovery rate and MCL	130
Table 4.11 Comparison of the study and excluded populations by clinical status	143
Table 4.12. Comparison of the study and excluded populations in the first MST application by age and gender	144
Table 4.13. Comparison of the study population and emigrants by age and gender	144
Table 4.14. Comparison of the study and the refusal group at the second MST survey	144
Table 4.15 Previous relevant studies of leishmaniasis epidemiology in Central and South America	147
Table 5.1 Species composition in the Opon focus	167
Table 5.2 Sandfly abundance measured by man-landing collections in four habitat types, two sites/habitat and 18 collections/site (18:00-20:00 hours): Seasonal variation study in San Pedro	168
Table 5.3 Sandfly abundance measured by man-landing collections in four habitat types, one site/habitat and three collections/site (18:00-06:00 hours): Nocturnal activity study in Santa Sofia	171
Table 5.4 Indoor collections of sandflies using CDC light traps in 13 villages (114 night - traps)	175
Table 5.5 Natural infection of sandflies in the Opon focus	178
Table 5.6 Comparisons between total nocturnal biting activity of sandflies: before and after “bed-time” (22:00 hours)	183
Table 5.7 The proportion of houses containing sandflies according to villages	185
Table 5.8 Distribution of sandfly species by village	185
Table 5.9 Sandfly activity inside houses according to surrounding vegetation type: Parameter estimates, standard errors and r^2 for the minimal adequate models generated by multiple regression analyses	188

Table 5.10 Sandfly activity inside houses according to the extent of surrounding pasture land:
Parameter estimates, standard errors and r^2 for the minimal adequate models generated by
univariate regression analyses 189

Table 5.11 Sandfly activity inside houses vs incidence and prevalence Parameter estimates, standard
errors and r^2 for the minimal adequate models generated by multiple regression analyses 190

Table 5.12 Natural infection rates in Colombian sandflies which are proven or suspected vectors of *L.*
panamensis 206

Table 5.13 Relationship between light trap and human catches of sandflies in four studies in Andean
Countries 208

Table 5.14 Seasonal variation studies of different sandfly species previously reported in Central and
South America 231

LIST OF FIGURES

	Page.
Figure 1.1 Some well researched LCL foci in Central and South America referred in the text	11
Figure 1.2 Sloth nails and skulls collected by farmers in Opon area	12
Figure 1.3 Distribution of LCL in Colombia	16
Figure 1.4 Distribution of LCL in Santander Department	21
Figure 1.5 Distribution of LCL in Landazury Municipality	22
Figure 1.6 Incidence of LCL in Colombia, 1981-1995	23
Figure 1.7 Incidence of LCL in Santander, 1985-1996	23
Figure 2.1 Ecological characteristics of the Opon area	31
Figure 2.2 Localisation of the study villages in the Opon area, Landazury Municipality, Santander Department, Colombia	32
Figure 2.3 Household in Opon	33
Figure 2.4 Household surrounded by forest	33
Figure 2.5 Vegetation features up to 50 m around households	34
Figure 2.6 Vegetation features up to 800 m around households	34
Figure 2.7 Relationship between vegetation features: land use	35
Figure 2.8 Rainfall patterns in the Opon focus, 1996	36
Figure 2.9 Frequency of rainfall in the Opon focus, 1996	36
Figure 2.10 Minimum temperature pattern in the Opon focus, 1996	37
Figure 2.11 Maximum temperature pattern in the Opon focus, 1996	37

Figure 2.12 Relative humidity pattern in Opon focus, 1996	38
Figure 2.13 Site on the arm where MST was applied	42
Figure 2.14 Diagram of the epidemiological study	44
Figure 2.15 Diagnostic methods used in Opon	48
Figure 3.1 Positive results of PCR in samples from LCL patients	61
Figure 3.2 PEPD patterns in parasites from Opon (IEA)	61
Figure 3.3 The relationship between the observed and fitted number of scars according to the MAM	70
Figure 3.4 Probability of lesions on the head by age	77
Figure 3.5 Probability of scars on the head by age when infected (in regions 1 and 2)	77
Figure 3.6 Probability of lesions on the arms by age	78
Figure 3.7 Probability of scars on the legs by gender (in regions 1 and 5)	78
Figure 3.8 Probability of scars on the legs by gender (in regions 1 and 5)	79
Figure 3.9 The rate of suspected metastasis	85
Figure 4.1 Population Structure	100
Figure 4.2 Distribution of excluded and study populations in Opon	103
Figure 4.3 Frequency distribution of MST induration sizes amongst L-	106
Figure 4.4 Frequency distribution of MST induration sizes amongst L+	106
Figure 4.5 Age prevalence curves of infection by gender	111
Figure 4.6 MST conversion rate by age	113
Figure 4.7 Household risk factors for infection: the relationship between the extent of pasture land surrounding a house and the proportion of m+	116
Figure 4.8 Village risk factors for infection: the relationship between vegetation coverage and cumulative prevalence in 11 villages	119
Figure 4.9 Village risk factors for infection: the relationship between vegetation coverage and incidence of infection in 11 villages	122
Figure 4.10 Age prevalence curves of clinical infection by gender	126
Figure 4.11 The relationship between age and the proportion of infection which are subclinical	127
Figure 4.12 The relationship between incidence of new lesions and MST size, clinical status and age	131

Figure 4.13 The relationship between the loss of MST responsiveness and both MST size and clinical status	133
Figure 4.14 The annual incidence rates of secondary lesions according to time since the primary lesion	134
Figure 4.15 Monthly incidence of primary leishmaniasis cases	136
Figure 4.16 Monthly incidence of recurrent leishmaniasis cases	137
Figure 4.17 Personal risk factors for MCL	139
Figure 5.1 Nocturnal activity of <i>Lu. yuilli</i>	180
Figure 5.2 Nocturnal activity of <i>Lu. hartmanni</i>	180
Figure 5.3 Nocturnal activity of <i>Lu. gomezi</i>	181
Figure 5.4 Nocturnal activity of <i>Lu. quasitowsendi</i>	181
Figure 5.5 Nocturnal activity of <i>Lu. trapidoi</i>	182
Figure 5.6 Cumulative biting of sandflies during the night (as a proportion of the total night's activity)	182
Figure 5.7 The relationship between indoor abundance of <i>Lu. gomezi</i> and household incidence rate	191
Figure 5.8 The relationship between indoor abundance of <i>Lu. trapidoi</i> and household prevalence (m+)	192
Figure 5.9 The relationship between an entomological index, incorporating the mean village abundance of both <i>Lu. trapidoi</i> and <i>Lu. gomezi</i> and the village incidence rate	193
Figure 5.10 The relationship between the mean village abundance of <i>Lu. trapidoi</i> and village prevalence (m+)	194
Figure 5.11 Seasonal variation of <i>Lu. hartmanni</i>	198
Figure 5.12 Seasonal variation of <i>Lu. quasitowsendi</i>	199
Figure 5.13 Seasonal variation of <i>Lu. trapidoi</i>	199
Figure 5.14 Relationship between rainfall frequency and the subsequent abundance of <i>Lu. quasitowsendi</i>	200
Figure 5.15 Seasonal variation in endophagic activity of sandflies in the Opon focus	200
Figure 5.16 Nocturnal activity patterns of <i>Lu. trapidoi</i> previously reported in Central and South America	225
Figure 5.17 Nocturnal activity patterns of <i>Lu. gomezi</i> previously reported in Central and South America	227

Figure 5.18 Nocturnal activity patterns of *Lu. hartmanni* and *Lu. yuilli* previously reported in Central
and South America 228

Figure 5.19 Seasonal variation studies of *Lu. trapidoi* previously reported in Cental and South
America 232

Figure 5.20 Seasonal variation studies of *Lu. gomezi* previously reported in Cental and South America 235

Figure 5.21 Seasonal variation studies of *Lu. hartmanni* previously reported in Cental and South
America 236

ABBREVIATION LIST

λ	force of infection
α	proportion of conversions associated with lesions
ρ	recovery rate
μ l	microlitter
6PGD	6 phospho gluconate dehydrogenase
a.s.l.	above sea level
ANOVA	analysis of variance
BAP	basic attention plan (Colombian Ministry of Health)
C	culture (C+ culture positive, C- culture negative)
C.I.	confidence Intervals
CDC	Centers for Disease Control and Prevention miniature light traps
CMI	Cell mediate immune response
CX	cross sectional studies
D.F.	degrees of freedom
DCL	diffuse cutaneous leishmaniasis
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide tri-phosphate
EDTA	ethylenediaminetetraacetic acid
ES	esterase

FW	follow up studies
G	Giemsa
GPI	glucose-phosphate isomerase
H	Kruskal-Wallis
H+	Spontaneous healing
H-	Chronic lesions
IEA	isozyme electrophoresis
<i>L</i>	<i>Leishmania</i>
I+	proportion of a particular sub-population who belong to L+
I-	proportion of a particular sub-population who belong to L-
L+	number of persons with present or past leishmaniasis lesions
L-	“healthy” population
LCL	localised cutaneous leishmaniasis
LSHTM	London School of Hygiene and Tropical Medicine
<i>Lu.</i>	<i>Lutzomyia</i>
m	meters
m+	proportion of a particular sub-population who belong to M+
m-	proportion of a particular sub-population who belong to M-
M+	positive MST
M-	negative MST
MCL	mucocutaneous leishmaniasis
ME	malic enzyme
ME	Microscope examination

mM	milimolar
mm	millimetres
MOH	Ministry of Health (Colombia)
MPI	mannose phosphate isomerase
MST	Montenegro skin test
N	North
n	number of people or sandflies in a population
ND	not done
NH	nucleoside hydrolase
NLCP	National Leishmaniasis Control Program
NNN	novy, McNeal and Nicolle medium
NW	new World
OR	Odds ratios
P	P value in the test of significance
P+	parasitological positive result
P-	Parasitological negative result
PBS	phosphate buffered saline
PCR	polymerase Chain Reaction
PEPD	proline dipeptidase
PGM	phosphoglucomutase
pmol	picomol
R	relative pathogenicity
r^2	correlation index in logistic regression
RH	relative humidity
RR	relative Risk
SDS	sodium dodecyl sulphate

spp	any parasite or reservoir especies
VL	visceral leishmanisis
W	West
WHO	World Health Organisation

1. INTRODUCTION

1.1 THE NATURAL HISTORY OF LEISHMANIASIS

American cutaneous leishmaniasis is a human disease, which describes a wide spectrum of clinical and immunological manifestations, including localised cutaneous leishmaniasis (LCL), mucocutaneous leishmaniasis (MCL) and diffuse cutaneous leishmaniasis (DCL) (Tapia et al., 1994). The evolution of disease in this spectrum is dependent upon a complex set of interactions between signalling properties of the epidermis (Tapia et al., 1994) and events associated with cell-mediated immunity (Curry et al., 1994). Leishmaniasis is caused by intracellular protozoan parasites of the genus *Leishmania* and is transmitted to humans by the bite of a small percentage of the 400 species of phlebotomine sandflies described to date in America (CIPA group, 1993). The sandflies become infected when taking blood from a reservoir host, which can include rodents, sloths, marsupials, dogs (Ashford, 1996) and probably humans (Montoya et al., 1990).

LCL is restricted to well-defined skin lesions because a Th1-like immune response is mounted by activated CD4⁺ helper T (TH) cells, including delayed-

type hypersensitivity (reflected by the response to a Montenegro Skin Test, MST) and macrophage activation. All *Leishmania* spp. listed in Table 1.1, as well as *L. chagasi* (Oliveira et al, 1986), have been associated with LCL. *L. braziliensis* infection is typically associated with more severe LCL pathology than *L. panamensis*, as it generally leads to a more protracted duration of disease and to larger cutaneous lesions. The mean MST induration size also tends to be greater following *L. braziliensis* infection (even after controlling for lesion duration, type and size) (Saravia et al, 1989). However, recurrences are thought to be more frequent following *L. panamensis* infection (Weigle et al, 1993).

MCL is a hyperergic form of LCL with an exaggerated antigen-specific, cell-mediated immune response (CMI), but a low density of parasites (Tapia et al., 1994). MCL is also associated with increased antibody titers as well as a heightened cutaneous MST response (Saravia et al, 1989). Lesions are often chronic and progressive, relapsing after treatment. The most severe cases may be associated with mutilation, deterioration of the general state of health and even death, when there is profound compromise of the respiratory system. In a five-year prospective study in Brazil, MCL occurred in 2.7% of LCL patients (Jones et al. 1987). MCL with a positive CMI response is associated most commonly with *L. braziliensis*.

DCL is a progressive, anergic, non-ulcerative form of leishmaniasis, which reflects severe antigen-specific T cell deficiency of the infected host. In the epidermis, the principal problem is related to cytokine-mediated accessory signals that affect the function of antigen-presenting cells (Convit et al, 1993; Tapia et al , 1994). DCL is generally associated with species in the *L. mexicana* complex, although some isolates from DCL patients have recently been characterised as *L. panamensis* (Velez et al, 1994).

The natural history of LCL caused by *L. panamensis* in one focus has been described in detail based on the results of a follow-up study conducted in Tumaco, Colombia (Saravia & Weigle, 1996). The study reported that *L. panamensis* infections had low pathogenicity (as only 12% of infections caused clinical symptoms) but relatively high virulence (i.e. large lesions and frequent reactivations amongst patients). However, in a small cohort followed in Santander during a pilot study in 1991, *L. panamensis* infections appeared to be considerably more pathogenic than those reported in Tumaco. This result indicated the need for additional studies on this parasite species in order to classify its clinical impact on human infection throughout its geographic range. Thus, one aim of this project was to compare the natural history of *L. panamensis* infections in the Pacific coastal lowlands of Tumaco with those occurring in the inter-Andean Valleys (where Opon is located).

1.2 LEISHMANIASIS AS A PUBLIC HEALTH PROBLEM IN SOUTH AMERICA

The world population at risk of leishmaniasis has been estimated by the World Health Organisation (WHO, 1995) as 350 million persons belonging to 88 countries, with a world prevalence of ca. 12 million. The world-wide annual incidence of the different clinical forms of leishmaniasis is estimated as ca. 1.5-2 million new cases/year (1-1.5 million cutaneous leishmaniasis; 500,000 cases of visceral leishmaniasis) (Desjeux, 1996) excluding "(1) cases who have no access to medical facilities, (2) misdiagnosed cases, and (3) cases that are seen clinically but not reported" (Ashford et al, 1992). Hence, this figure is likely to be a gross underestimate.

In the New World the estimated incidence of LCL is ca 60,000 person/year. LCL is endemic in 20 of the 22 continental countries, and in two Caribbean islands. The mean number of annual cases reported during the period 1986-1990 gives an idea of the distribution of leishmaniasis in American countries: Brazil had the highest annual incidence (67,500 cases reported) followed by the Andean and Central American countries (total of 57,200 cases). Only 1,500 cases were reported in Argentina, Paraguay, USA and Mexico during the same period (Desjeux, 1991 and 1992).

A significant increase in the number of reported cases of leishmaniasis has become apparent in recent years in the New World. For example, 2,000 cases were reported in the northern states of Brazil in 1980; nine years later the annual number of cases reported had risen to 9,000 (Desjeux, 1991). Similarly, in Peru, the average incidence ranged between 6.8 and 8.8/ 100,000 person/year in the period 1951 -1979, while in the 1980s the annual incidence averaged ca. 14.5/100,000 person/year (Rodriguez, 1992). The increase in reported cases could in part be due to the recent status of leishmaniasis as a notifiable disease, as there is undoubtedly now greater medical knowledge, drug availability and active search of cases (Scorza, 1985; Desjeux, 1992).

Several factors related to the ecological, economic and political changes in Latin-American countries could also have caused an increase in the public health importance of leishmaniasis. In Venezuela, the major leishmaniasis foci have been displaced from agricultural fields to sub-urban areas, where the population has concentrated since the boom which resulted from oil exploitation (Scorza, 1985). There is also circumstantial evidence that *Leishmania* parasites may have adapted to a peri-domestic reservoir, *Didelphis marsupialis* (Scorza et al, 1984) or even to a domestic reservoir (dogs or equines) (Ashford, 1996). In addition, the shortage of arable land, and the problem of overpopulation in the high plateaux of some Andean Countries, has led to increasing settlements in the tropical plains where the risk of transmission is frequently higher (WHO, 1995). In Colombia, as in Peru (Davies et al,

1995a), the political decision to terminate the insecticide spraying regime of the Malaria Control Campaign has also been associated with the resurgence of leishmaniasis (WHO, 1995; Cepeda, 1997).

In general, the main site of leishmaniasis transmission in Central and South America has changed from the sylvatic to the domestic environment (including indoors). Transmission is often highest in new settlements in endemic areas, where new agro-industrial projects have recently been developed. However, in the last national report available from the Colombian Ministry of Health, leishmaniasis is still a predominant disease of males within an age range of 15 to 44 years, and infection is still frequently associated with forest activities. This reported epidemiological pattern could be biased because the great majority of cases are identified by passive search. Therefore, the data could be biased in favour of those with a greater probability of attending distant hospitals in order to receive treatment. Large number of cases will be never be registered, and children are especially likely to be excluded, as they are often treated with “traditional medicines” or commercially bought Glucantime® (typically given in doses too low to be effective), and are therefore not reported to the central authorities. Thus, the risk factors for leishmaniasis in Colombia cannot be estimated satisfactory from the Ministry of Health data, and there is a requirement to carry out a series of large scale

cohort studies, in order to provide a rational basis for deciding appropriate policies for treatment and prevention.

1.2.1 *Leishmania* species distribution in America

The distribution of the most *Leishmania* species in Central and South America is closely related to specific ecological conditions. The isolation and classification of parasites from humans, vectors and reservoir hosts, throughout the New World, has also identified strict geographical ranges for most *Leishmania* species. In general, *L. panamensis*, *L. guyanensis*, *L. mexicana*, *L. amazonensis* and *L. peruviana* are restricted to more defined environments, whereas *L. braziliensis* is more widespread (Table 1.1).

L. panamensis is distributed in some Central America countries, in Colombia and in Ecuador. It is absent in Belize, rare in Guatemala and El Salvador, but relatively frequent in Honduras, Costa Rica and Panama. The situation is not clear in Nicaragua. In South America, *L. panamensis* extends its influence through the inter-Andean valleys in Colombia (Cauca river and Magdalena river valleys: see below) and the tropical lowland forests of Colombia and Ecuador (on the Pacific coast) (Belli et al, 1993; Grimaldi et al, 1989; Corredor et al, 1990; WHO, 1990; Nichols, personal communication). The Pacific coastal lowlands of Ecuador and Colombia have a similar fauna

and flora as Central America (Corredor et al., 1990). In this area is located Tumaco Municipality (Colombia), where the only previous reported cohort study of *L. panamensis* transmission was carried out (Figure 1.1) (Weigle et al, 1993).

The reservoir host of *L. panamensis* is thought to be the two-toed sloth, *Choloepus hoffmanni* (Ashford, 1996), on the basis of frequent parasite isolation and its geographical distribution. The geographical distribution of *L. panamensis* coincides clearly with the distribution of *C. hoffmanni* (Herrer and Telford, 1969), except that the sloth is found in Guyana and Brazil (Emmons, 1994), where *L. panamensis* is absent. In Venezuela, the sloth is present and *L. panamensis* has only rarely been reported. Hence, the geographical distribution of *L. panamensis* coincides better with the regions defined by the overlapping ranges of *C. hoffmanni* and *Lu. trapidoi*, the presumed principal vector of this parasite (see Chapter 5). In Opon, where this project was carried out, *C. hoffmanni* is found in large numbers, and is hunted on a large scale. This is apparent from the huge collections of sloth nails and skulls kept by the farmers as trophies of their hunting practices (Figure 1.2)

L. braziliensis is the most widespread *Leishmania* species in Latin-America. It is the predominant aetiological agent of LCL in the northern countries of Belize and Guatemala, but is relatively rare in Panama and Costa Rica (Belli et al., 1993). In Colombia, Venezuela, Ecuador, and Peru *L.*

braziliensis becomes an important public health problem. The main foci are limited to the western Andean region in Venezuela (Trujillo, Merida and Tachira) and to the Catatumbo River Valley in north-western Colombia (Nichols, personal communication; Maingon et al., 1994). *L. braziliensis* is the principal causative agent of cutaneous leishmaniasis in Brazil and Bolivia and is the only *Leishmania* species isolated from humans in Argentina and Paraguay.

Table 1.1 Recognised Leishmania species causing human diseases in the New World

Parasite	Known geographic distribution
<i>L. braziliensis</i>	Argentina, Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Panama, Paraguay, Peru, Venezuela.
<i>L. peruviana</i>	Peru
<i>L. guyanensis</i>	Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, Venezuela
<i>L. panamensis</i>	Colombia, Costa Rica, Ecuador, Honduras, Nicaragua, Panama, Venezuela
<i>L. lainsoni</i>	Brazil, Peru
<i>L. naiffi</i>	Brazil
<i>L. colombiensis</i>	Colombia, Panama, Venezuela
<i>L. shawi</i>	Brazil
<i>L. mexicana</i>	Belize, Colombia, Costa Rica, Dominican Republic, Ecuador, Guatemala, Honduras, Mexico, Panama, United States, Venezuela
<i>L. amazonensis</i>	Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Panama, Peru Venezuela
<i>L. venezuelensis</i>	Venezuela

* Taken from Grimaldi and Tesh, 1993

L. mexicana has been isolated in the United States, Central America, Colombia and Venezuela. In Central America, it is relatively common in Guatemala (Desjeux et al., 1991), Belize and El Salvador (Belli et al., 1993), and has some public health importance in Venezuela (Maingon et al., 1994) and Colombia (Corredor et al 1990). In the northern countries of South America, *L. mexicana* is distributed through the Pacific coastal lowlands (like *L. panamensis*), extending its influence through the Venezuelan-Colombian border at the North West of Venezuela (Tachira state) and the North East of Colombia (Norte de Santander Department) (Maingon et al, 1994; Grimaldi et al., 1989).

L. guyanensis has been isolated in Brazil, Colombia, Peru, French Guiana, Guyana and Surinam, and appears to be restricted to the Amazon basin. *L. amazonensis* is also found in the Amazon basin, and has been isolated in Venezuela, Peru, Ecuador and Bolivia. (Grimaldi et al, 1989). *L. peruviana* is restricted to the inter-Andean Valleys (800-3000 m a.s.l.) and western slopes of the Andes in Peru (Llanos Cuentas, 1993); whereas *L. colombiensis* has been isolated only in Venezuela, Colombia and Panama from a few human cases and from the sandfly *Lu. hartmanni* in tropical rain forest habitats (Kreutzer et al., 1991)

Figure 1.1 Some well researched LCL foci in Central and South America referred in the text



Figure 1.2 Sloth nails and skulls collected by farmers in Opon area



1.3 LEISHMANIASIS AS A PUBLIC HEALTH PROBLEM IN COLOMBIA

1.3.1 Introduction

The National Health System of Colombia (South America), was restructured in 1993 on the basis of a new law (Law number 100), within which the “Basic Attention Plan” (BAP) was created to curb tropical diseases. BAP is currently funded by the Ministry of Health and is operated throughout Colombia by each Departmental Health Offices. Santander Department (the focus of this thesis) is located in north-eastern Colombia on the eastern side of the Andean mountains (see below). This region is characterised by a rich fauna and flora and little climatic variation throughout the year. Tropical diseases prevalent in Santander include leishmaniasis, Chagas disease, dengue and malaria; and are all managed by the “Vector Borne Disease Control Unit” (BAP-Santander), which is located in the Santander Health Office. This Unit inherited all the equipment and staff, belonging to the former National Malaria Control Program, which was ended in 1995.

The BAP policies are generated by the Colombian Ministry of Health which has created a series of recommendations in the past four years for the control of the major endemic vector-borne diseases in Colombia. The aims of

the National Leishmaniasis Control Program (NLCP), which was set up by BAP are: (1) to study the parasites, vectors, and reservoir host(s) involved in leishmaniasis transmission, and (2) to study the risk factors for transmission which are associated with human behaviour. This theoretical frame work covers all the topics needed for the establishment of national control programs, but in practice research programmes are limited by inaccessibility of many isolated foci and by the small departmental budgets. One aim of this thesis is to evaluate the feasibility of carrying out the objectives of the NLCP in a typical isolated focus of leishmaniasis, and to make recommendations on the methodology most suitable for data collection and analysis.

In the following sections, the importance of leishmaniasis in Colombia is presented with respect to other Latin-American countries. Particular attention is paid to Santander Department in relation to the other Colombian departments.

1.3.2 The distribution of *Leishmania* species in Colombia

Colombia has unusual mountain formations that produce a variety of rainfall patterns, forming 23 distinct vegetative zones: These include desert bush, tropical moist forest (2000–4000 mm of rain per year), tropical wet forest (4000–8000 mm of rain per year), and tropical rain forest (over 8000 mm of rain per year) (Espinal et al., 1963). The geographical distribution of *Leishmania*

species in Colombia is related to the distribution of these ecological patterns, which can be classified into seven large regions: (1) the Atlantic and Pacific coast; (2) Amazonia and the eastern plains; (3) the Magdalena River Valley; (4) the Catatumbo River Basin; (5) the Cauca River Valley; and (6) Central Andean Massif (Figure 1.3).

When examining 670 isolates (including 340 isolates previously reported by Corredor et al, 1990) from patients, sandflies and reservoirs by isoenzyme electrophoresis, Nichols (personal communication) concluded that *L. panamensis* was the most frequently isolated parasite in Colombia (309/670, 46%), followed by *L. braziliensis*. However, *L. braziliensis* is the most widely distributed species, while *L. panamensis* appears to be confined mainly to the western half of the country in both the inter-Andean valleys and in the Pacific coast (Figure 1.3). *L. guyanensis* has been isolated from patients and sandflies (*Lu. umbratilis*) in the Amazon basin, in south-eastern Colombia. *L. amazonesis* is also present in the Amazon basin and on the west slope of the eastern Andes, having been isolated from patients with LCL and Diffuse Cutaneous Leishmaniasis (DCL) (1% of the 670 strains). A small number of isolates of *L. mexicana* were also detected, being restricted to the lowlands Pacific Coast (Figure 1.3)

Figure 1.3 Distribution of LCL in Colombia



1.3.3 Regional variation in the annual Incidence of LCL in Colombia.

LCL has long been prevalent in Colombia, as indicated by Pizarro's reference to indigenous people with nasal mutilations. Later on, Gomez reported a case in 1872, calling it "puercas" or "marranas". In 1893, Mateus and Francisco made the first clinical description of a patient, who came from the Department of Santander, naming the disease "bubon de Velez", and the *Leishmania* parasite was first observed and described in Colombia by Rodriguez in 1929 (Werner & Barreto, 1981).

Ward (1975) concluded that 2,000 cases were probably seen in Colombia between 1948 - 1955. However, Werner and Barreto (1981) only reported 283 cases in their review of the distribution of confirmed leishmaniasis cases in Colombia on the bases of reports published between 1929 and 1979. The Colombian Ministry of Health (MOH) reported 930 cases in 1976; and in 1980 the MOH instituted an obligatory register for leishmaniasis cases. The reported LCL incidence has since then increased year by year, with a peak of 64 cases/100,000 person-years in 1993 (Figure 1.6). From the total leishmaniasis cases reported between 1990-1995, 95.7% were cutaneous, 3% mucocutaneous and only 1.3% visceral (Cepeda, 1997).

From six of the seven ecologically defined regions (i.e. excluding the Central Andean Massif), autochthonous LCL cases have been reported in all Colombian departments, with the exception of the Department of Atlantico and the islands of San Andres and Providencia (Werner & Barreto, 1981; Colombia Ministry of Health, 1986; Cepeda, 1993, 1997). The highest regional incidence in 1995 occurred in the Catatumbo River basin, followed by the two inter-Andean valleys (Magdalena and Cauca Valleys) (Figure 1.3). The Department of Santander, where this project was carried out, had the fourth highest number of reported LCL cases in Colombia (Table 1.2).

1.3.4 Variation in the annual incidence of LCL in Santander Department, according to Municipalities

Santander comprises 86 "Municipalities", organised into six regions, called "Provincias" (Figure 1.4). Four of the "Provincias" are principally located in an inter-Andean valley (Magdalena valley) at 100 m a.s.l., and on the western slope of the East range of the Andean mountains (up to ca. 1,200 m a.s.l.). The four Provincias are: Soto, Mares, Socorro and Velez. The other two Provincias (San Gil and Garcia Rovira) are located exclusively in mountainous areas at high altitudes (maximum: 4,000 m a.s.l.) (Figure 1.4). The reported incidence rates in Santander have been increasing since 1991, when the active search of cases started in Landazury Municipality during a pilot

study carried out prior to this thesis (Figure 1.7). All reported cases in Santander are confined to 30 of the 86 municipalities, and 93% of the cases are located in three Provincias: Soto, Mares and Velez (S.S.S., 1996). In 1996, incidence rates over 500/100,000 person-years were found in four Municipalities: Landazury, El Carmen, Sucre and San Vicente, which are all located in the South-West of Santander Department (Figure 1.4). These four municipalities are situated in the foothills of the mountains, between the Magdalena valley and the East Mountain Range, at an average altitude of 750 m a.s.l. (range: 300-1200 m a.s.l.) (Figure 1.4).

Landazury has the highest reported leishmaniasis incidence of any municipality in Santander (1,368/100,000 persons-years reported in 1996). The capital is located 286 Km from Bucaramanga (the departmental capital), lies at an altitude of 1,100 m a.s.l., with an average temperature of 23 °C (see Materials and Methods). The municipality is divided into nine “districts” with a total population of 13,000 inhabitants. Between 1991 and 1993, 367 cases were detected by passive search and diagnosed by parasite detection in thin smears made from superficial scrapings of leishmaniasis ulcers. During this period, the annual incidence rate of LCL in the nine districts ranged from 0/1000 person-year (La India) to 21.7/1000 person-year (Chorolo) (Figure 1.5). Between 1995-1997 Chorolo District presented an average incidence rate of

28/1000 person-year (by passive search of cases) whilst in the same period the incidence rate in the Opon area (was twice as high) (see section 4.2.3).

Table 1.2 Distribution of cutaneous leishmaniasis in Colombia by regions

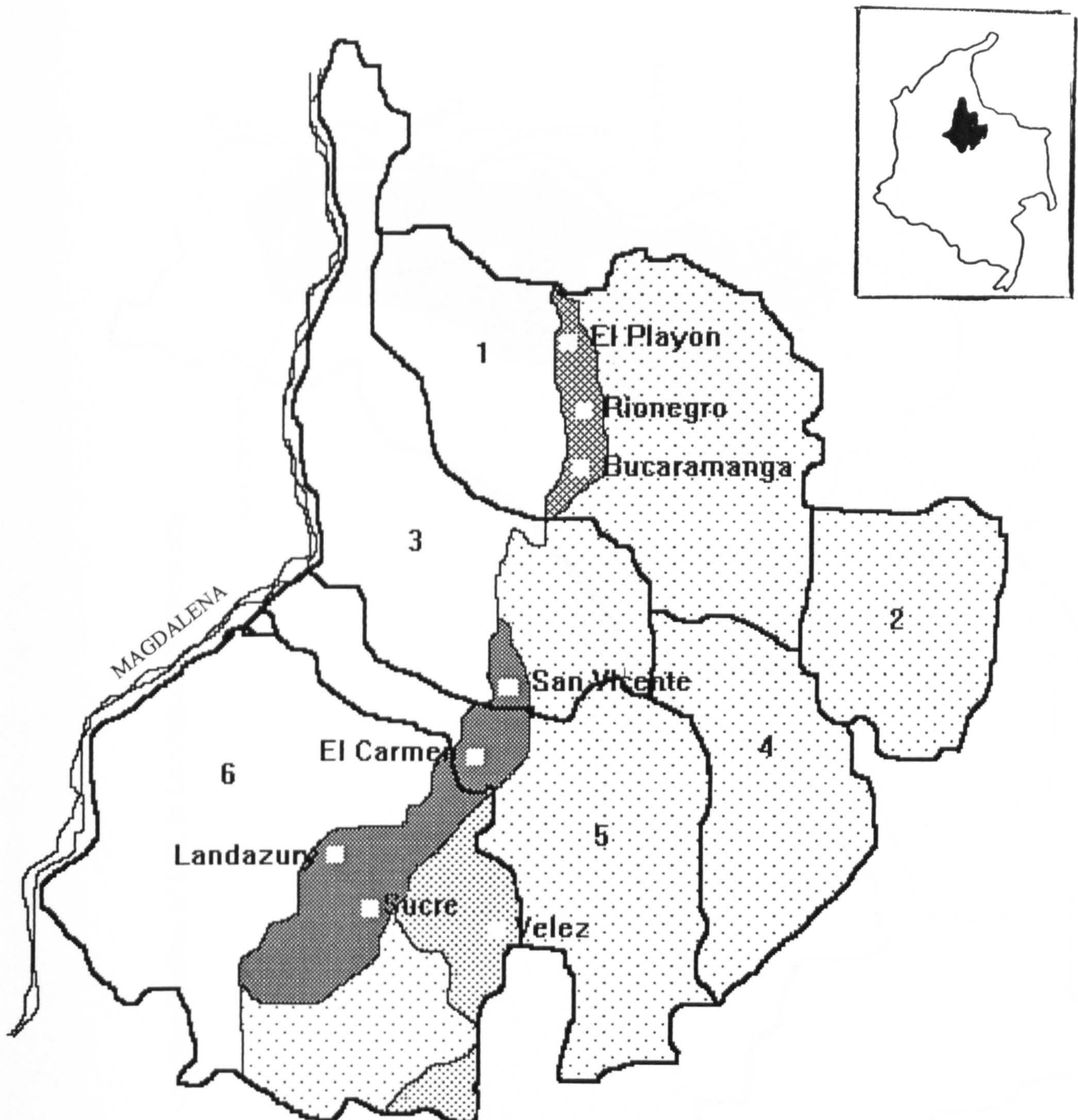
REGIONS / DEPARTMENTS	POPULATION AT RISK (rural pop.)	AVERAGE CASES/YEAR 1990 - 1994	AVERAGE INCIDENCE 1990-1994 X 100.000	NUMBER CASES 1995	INCIDENCE 1995 X 100.000
ATLANTIC COAST					
Guajira	37,237	40.8	109.6	10	26.9
Cesar	89,566	94.8	105.8	68	75.9
Magdalena (Santa Marta)	118,694	65.6	55.3	27	22.8
Atlantico (Barranquilla)	1,026,352	3	0.3	24	2.3
Bolivar	180,806	201.8	111.6	223	123.3
Sucre	122,310	330.6	270.3	337	275.5
Cordoba	268,331	128.4	47.9	197	73.4
TOTAL	1,843,296	865.0	46.9	886	48.1
PACIFIC COAST					
Choco	190,735	369.8	193.9	341	178.8
Cauca	334,171	46.2	13.8	76	22.7
Nariño	251,506	281	111.7	217	86.3
TOTAL	776,412	697.0	89.8	634	81.7
MAGDALENA RIVER VALLEY					
Santander	236,164	338.2	143.2	435	184.2
Cundinamarca	276,420	122.4	44.3	73	26.4
Tolima	192,694	192	99.6	119	61.8
Boyaca	68,553	31.4	45.8	68	99.2
Huila	81,646	161.8	198.2	590	722.6
TOTAL	855,477	845.8	98.9	1285	150.2
RIO CAUCA VALLEY					
Antioquia	786,887	1081.8	137.5	1,042	132.4
Caldas	187,913	460.8	245.2	363	193.2
Risaralda	8,677	230.8	2,659.9	120	1383.0
Quindio	5,635	7.4	131.3	12	213.0
Valle del cauca	158,847	128	80.6	84	52.9
TOTAL	1,147,959	1908.8	166.3	1621	141.2
CATATUMBO RIVER VALLEY					
Norte de Santander	286,791	938.4	327.2	998	348.0
AMAZONIA AND THE EASTERN PLAINS					
Arauca	52,085	17.8	34.2	42	80.6
Casanare	31,139	5.6	18.0	10	32.1
Vichada	10,272	9.4	91.5	13	126.6
Meta	189,757	88	46.4	77	40.6
Guainia	7,912	5.2	65.7	8	101.1
Caqueta	129,090	160.6	124.4	261	202.2
Vaupes	9,701	7.6	78.3	4	41.2
Guaviare	54,080	63	116.5	56	103.6
Putumayo	70,759	46.6	65.9	95	134.3
Amazonas	14,302	20.6	144.0	34	237.7
TOTAL	569,097	424.4	74.6	600	105.4
GRAND TOTAL	5,479,032	5679.4	103.7	6024	110.0

Not specified

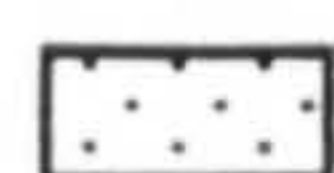
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Figure 1.4 Distribution of LCL in Santander Department

**PROVINCIAS**

1. Soto
2. Garcia Rovira
3. Mares
4. Socorro
5. San Gil
6. Velez

**Andes Mountains****INCIDENCE RATES****> 500/100,000 cases/man-years****100-500/100,000 cases/man-years****< 50/100,000 cases/man-years**

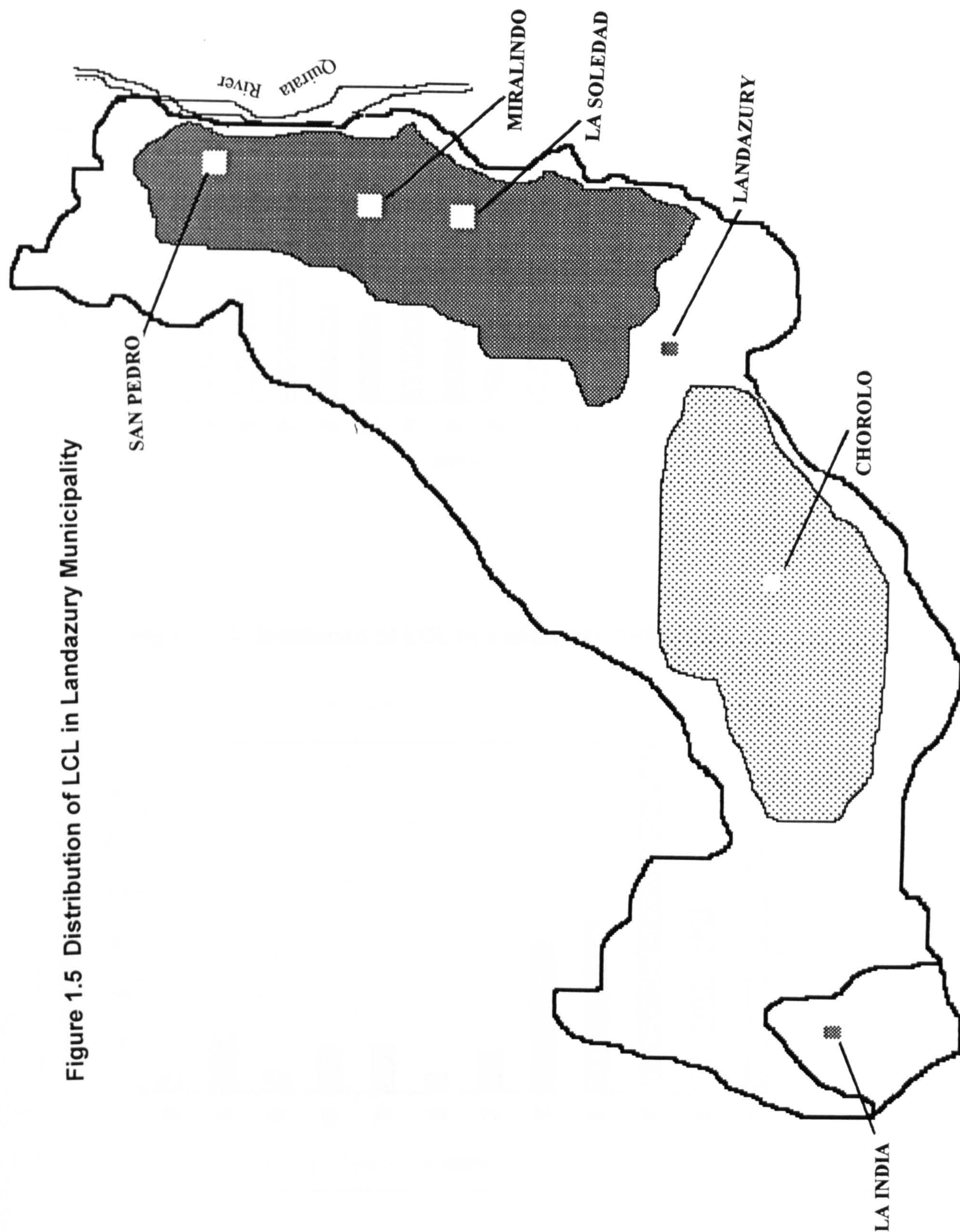
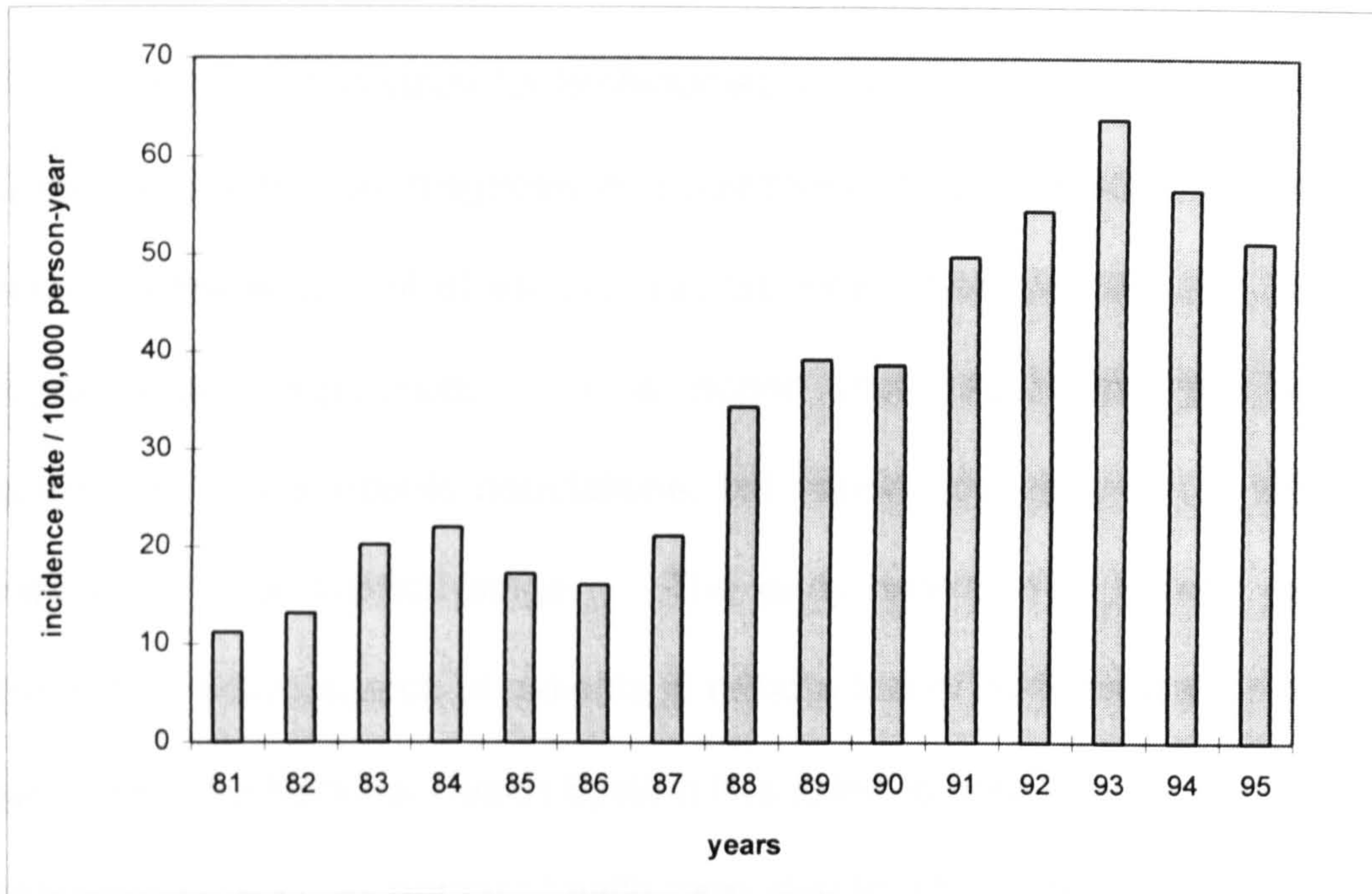
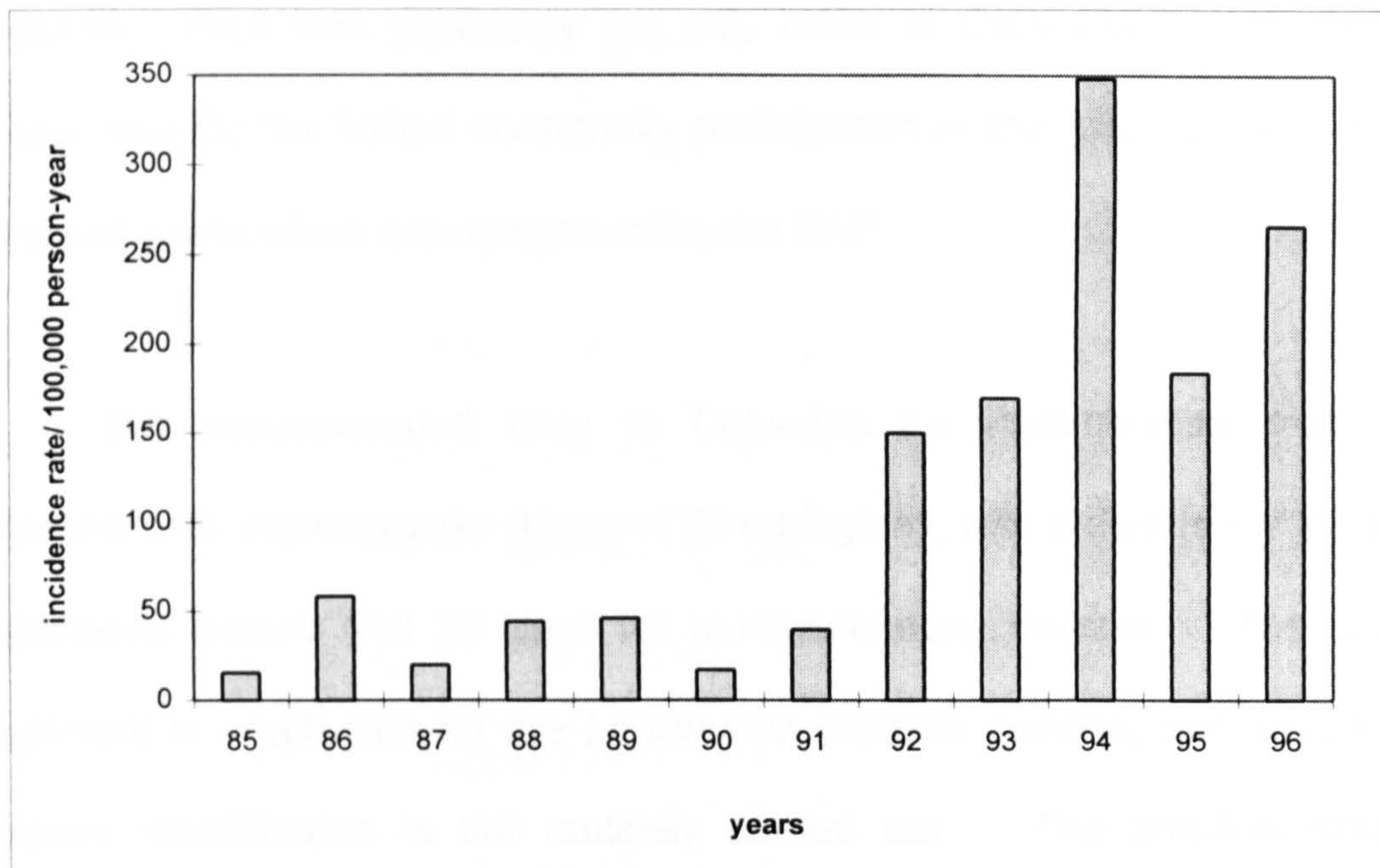


Figure 1.5 Distribution of LCL in Landazury Municipality

Figure 1.6 Incidence of LCL in Colombia, 1981-1995**Figure 1.7 Incidence of LCL in Santander, 1985-1996**

1.4 RATIONALE AND OBJECTIVES OF THE OPON FOCUS STUDY

The current strategy for leishmaniasis control in Colombia (NLCP, 1994) is (1) the early detection, diagnosis and treatment of active lesions; (2) the chemical and mechanical control of sandfly vectors which bite indoors; and (3) reservoir control where appropriate. It is hoped that future strategies will include vaccination of susceptible populations, but vaccine development is still at a very preliminary, experimental stage. The early detection of lesions can only be possible by active search of patients in remote foci of leishmaniasis. However, in these sites, the National Health System has failed to operate a number of essential programmes, such as primary health care, due to: (1) a great shortage of hospital staff for field work, as local political leaders (with the responsibility for these decisions under law 100) rarely include primary health care as a priority; (2) the decentralisation and departmental politicisation of the former Malaria Control Program, which was previously the only office in Colombia working in remote areas; and (3) the limited community participation in the establishment of those local net-works which are recognised by the BAP.

The recommended drug in Colombia for leishmaniasis treatment is Glucantime®: intramuscular doses of 20 mg/kg/day, with a duration of 20 days for cutaneous lesions and 28 days for mucocutaneous disease. The choice of treatment is unaffected by the *Leishmania* parasite species, not least because species identification is not routinely carried out. The principal criteria for

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treatment is that a case must be parasitologically positive, but clinically suspect cases may be treated even after a negative diagnostic test. The currently recommended diagnostic test is microscopic examination of lesion smears and histopathology. Microscopic examination of smears is carried out locally, i.e. at the nearest hospital to the patient, whereas histopathology is carried out at a regional health centre. If the first direct examination of a smear is negative, a second smear is made and examined. If a negative result persists, a biopsy has to be made in the local hospital, and sent to the central laboratory in the departmental capital. There may be a considerable delay before histopathology results are reported back to the local hospital. Normally, 15 days are required before a decision to treat a patient can be made exclusively on the basis of clinical diagnosis (i.e. in the absence of a positive parasitological diagnosis). During these 15 days, patients often return to their homes, and may purchase a sub-therapeutical dose of Glucantime®, which can be found on the black market. Hence, only a small proportion of patients are probably diagnosed and treated on the basis of histopathology or clinical diagnosis. In order to avoid problems associated with the black market, hospital staff provide patients with the required Glucantime in several batches. The patients take each batch home, where they administer it themselves (or with the help of a local health promoter), and then return to the hospital with their empty vials in order to pick up the next batch. Because the distance to a local hospital may be considerable, patients are often tempted to desert their course of treatment. Also the financial benefits of selling

their batches of Glucantime on the black market are a further incentive to stop treatment. Little is known yet about the extent to which drug resistance may be induced by the indiscriminate use of Glucantime®, which is a feature of these remote leishmaniasis foci.

Recommended vector control strategies include insecticide spraying indoors and in the peridomicile, where sandfly vectors are found in these environments. However, the efficacy of sandfly vector control in Colombia has only been evaluated following small scale trials of deltamethrin-impregnated bednets or curtains (Alexander et al, 1995b; CIDEIM, unpublished). With respect to MOH operational activity, insecticide spraying for LCL vectors is sporadic, and its effectiveness has never been evaluated either entomologically or epidemiologically. Insecticide spraying against LCL vectors has, for example, been carried out occasionally in Santander and Huila Departments. The insecticides used by the Ministry of Health include: Malathion, Fenitrothion, Propoxur, Deltamethrin, Lambdacyhalothrin and Permethrin. The insecticide application is carried out by trained staff with the appropriate equipment. At present, the staff of the former Malaria Control Program, who are trained for this purpose, are also responsible for insecticide spraying against *Aedes* and *Anopheles* mosquitoes and triatomine bugs. These latter activities may inadvertently reduce the incidence of leishmaniasis, where the different domestic vectors have overlapping distributions. There is little reliable information currently

available on the seasonal changes in abundance of sandfly vectors (except in a few well researched foci), so there is no clear strategy for selecting the appropriate time of the year (or the specific place) where insecticide should be applied. Indeed, the effectiveness of operational vector control programmes has never been measured satisfactorily. Mechanical control recommendations include the cutting down of small trees around houses and the removal of vegetal detritus from around houses. This control strategy is inappropriate where crops are grown up to the edge of houses, as in coffee and cacao plantations. There are no current recommendations on the control of putative domestic reservoirs for cutaneous leishmaniasis, and new research into LCL reservoirs is called for.

This thesis describes a field study of the dynamics of parasite transmission in humans at the Opon focus, combined with a study of the behaviour and ecology of the sandfly vector population. The transmission rate for human infections in the focus was measured using both cross-sectional and follow-up data, in order to estimate the real magnitude of the public health problem caused by leishmaniasis. The variable clinical response to infection was measured in relation to personal risk factors, in order to allow the targeting of the most susceptible groups in future control strategies, based on the early detection and treatment of cases. Evidence for sub-clinical infections, and for acquired protective immunity following either subclinical or clinical infections, was sought in the human population at risk in Opon, in order to provide information which might be pertinent to the development and testing of leishmaniasis vaccines in the future. Finally, the thesis focuses on

the risk factors for human infection, including the seasonal and temporal activity patterns of sandflies as well as changes in land use as a result of deforestation, in order to (1) determine the feasibility of interventions aimed at the prevention of leishmaniasis by reducing human-sandfly contact rates, and (2) to predict the long term epidemiological consequences of deforestation in the Colombian Andes.

The general objectives of the thesis were therefore as follows:

1. To provide an accurate estimate of the incidence of cutaneous leishmaniasis in the Opon focus, and to identify the parasites responsible.
2. To determine the demographic, seasonal, environmental and entomological risk factors for *Leishmania* transmission in the Opon focus, paying special attention to the effects of the widespread deforestation in this region.
3. To measure the variable human response to *Leishmania* infection in the endemic zone, and to identify factors that influence both the probability and severity of clinical symptoms
4. To formulate a practical approach for the study of leishmaniasis foci within the framework of the National Leishmaniasis Control Program.

2. MATERIALS AND METHODS

2.1 STUDY AREA

The study was carried out in 12 villages in the Opon area, Landazury Municipality, Santander Department, Colombia, South America. The villages are located ca. 290 km South-West of the departmental capital, Bucaramanga. It is a mountainous region (Figure 2.1), covering approximately 250 km², which is limited by the Quirata River (400 m a.s.l.) to the East, the "Cerro de Armas" mountain to the West, and two large cattle growing areas to the North and South (6°, 20' N; 73°, 43' W) (Figure 2.2). Most dwellings are built with wooden planks over wooden platforms, supported by stilts. The roofs are made of sliding corrugated iron, which can be moved during the day to expose the flat topped roof to the sun, where the farmers dry the cacao fruit (Figure 2.3). There are a total of 527 houses in the 12 villages, 331 of which are owned by people who constituted the study population (see Chapter 4)

The only access for transport is an unpaved road which passes through Miralindo, Santa Sofia, San Pedro and Tagual villages. Miralindo is the

larger village, the business centre for the community, from which a secondary road leads to the western villages of Plan de Armas, Las Delicias, La Dorada, Buenos Aires and Cucuchonal (Figure 2.2). Plan de Armas is the business centre for the last four villages. The principal economic activities are silviculture of tropical hard woods (in the rain forest), cattle ranching, and the cultivation of cacao (where the forest has been partially cut-down). A representative sample of 114/331 houses (see Chapter 4) were chosen to describe the characteristic surrounding vegetation coverage. Pasture land (and to a lesser extent cacao plantations) are typically found close to houses, so that the majority of land within 50m of each house is either pasture or cacao (Figure 2.3 and Figure 2.5a,b). In contrast, secondary forest is found within 50m of only 26% of the houses in this area (Figure 2.5c) and only a tiny percentage of houses are close to primary forest (Figure 2.5d). However, at greater distances from houses, the pattern of land use changes, with an increase in the coverage of cacao, secondary forest and primary forest and a decrease in pasture land (Figure 2.4 and Figure 2.6a-d). The relationship between land use and the percentage coverage with primary forest gives an indication of the ecological impact of a particular land use on the destruction of primary forest. For example, the impact of cacao plantations on the destruction of primary forest was less severe (Figure 2.7a) than the loss of primary forest associated with changing to pasture or non-permanent crops (which tend to be followed by secondary forest growth)

(Figure 2.7b,c). A negative impact was also observed for the relationship between cacao and pasture or secondary forest (Figure 2.7d,e).

In 1996, meteorological data (minimum and maximum temperature, relative humidity and rainfall) were recorded daily at the field station in San Pedro village. The annual rainfall was 2,099 mm , with two peaks between March to June and October to November (Table 2.1 and Figure 2.8). However, rainfall frequency (i.e. the proportion of days with some rainfall) only peaked once during the year: in April-May (Figure 2.9). The average minimum and maximum temperature was 19.8°C (Figure 2.10) and 33.3°C (Figure 2.11), respectively. The average relative humidity was 91.7% (Figure 2.12). A more detailed description of climatic patterns during the year is presented in Chapter 5.

Figure 2.1 Ecological characteristics of the Opon area



Figure 2.2 Localisation of the study villages in the Opon area, Landazury Municipality, Santander Department, Colombia

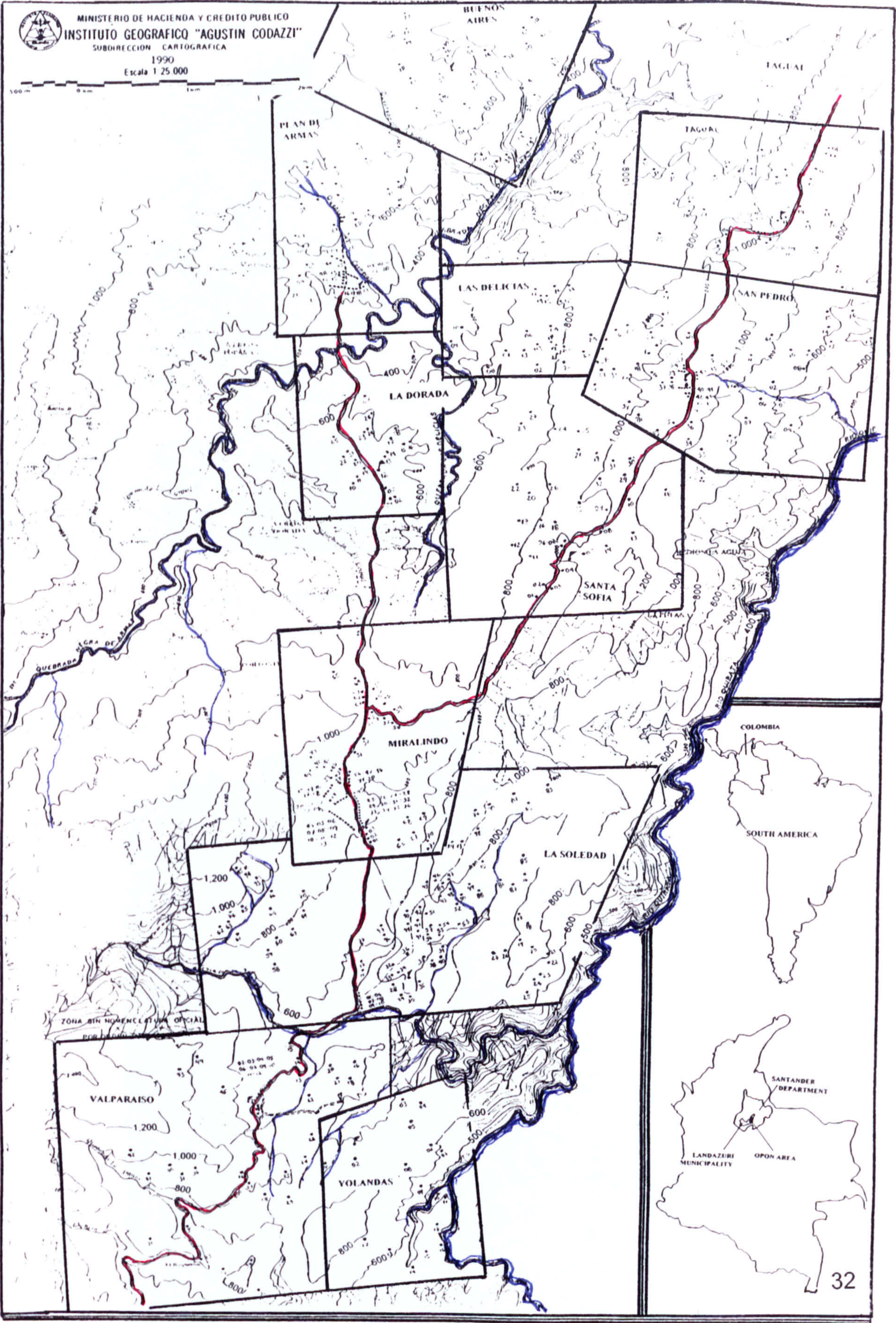


Figure 2.3 Household in Opon



Figure 2.4 Household surrounded by forest

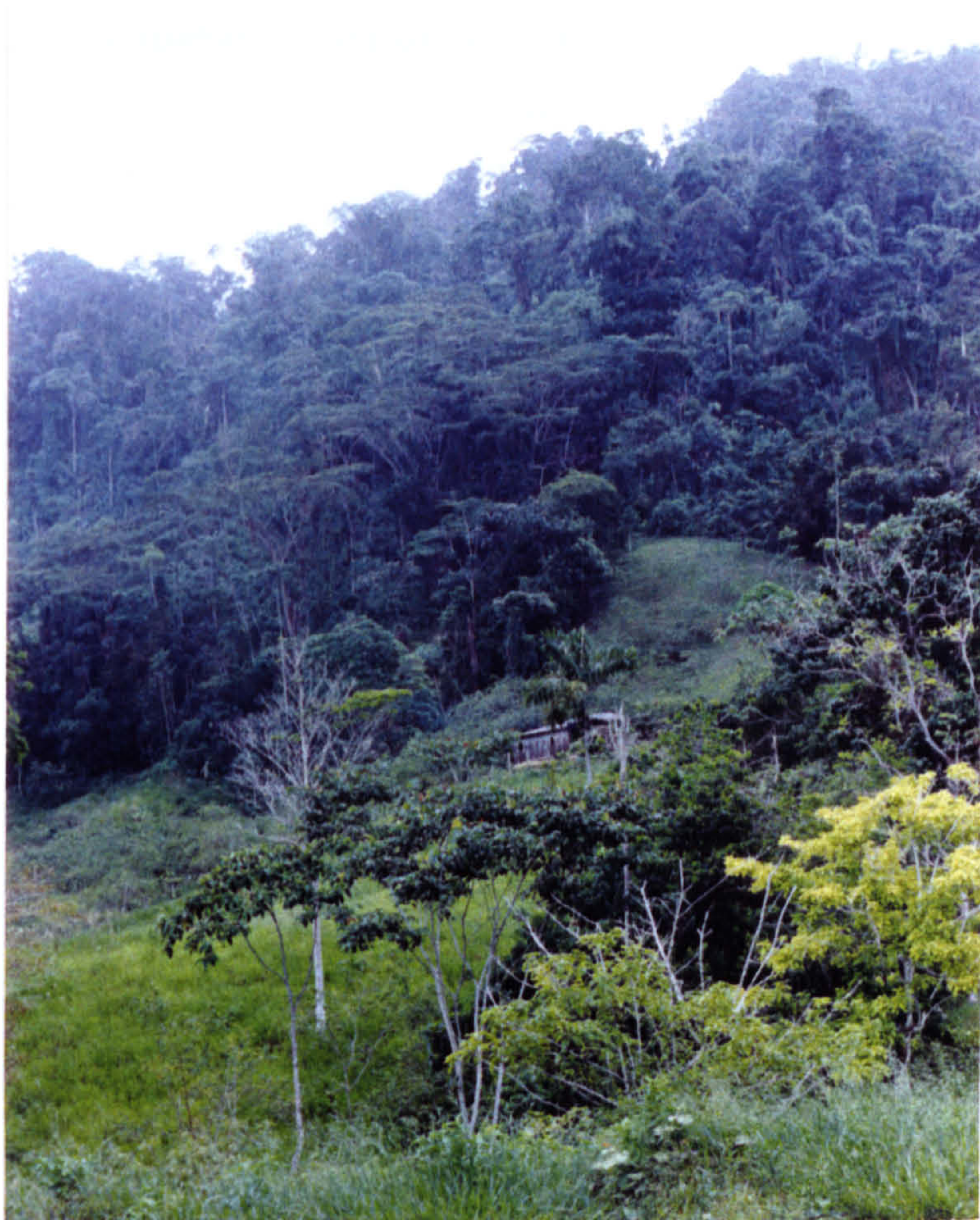


Figure 2.5 Vegetation features up to 50 m around households

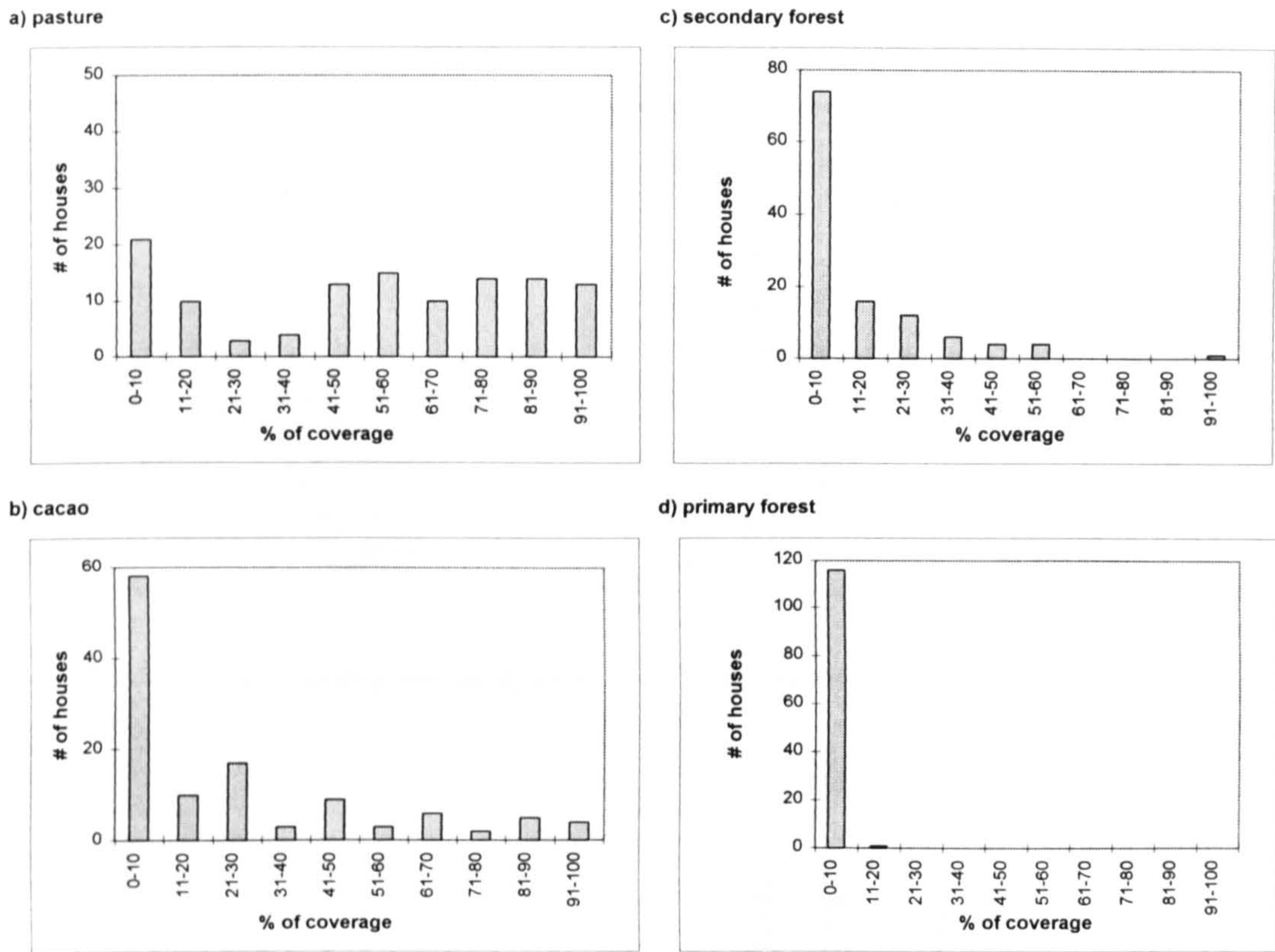


Figure 2.6 Vegetation features up to 800 m around households

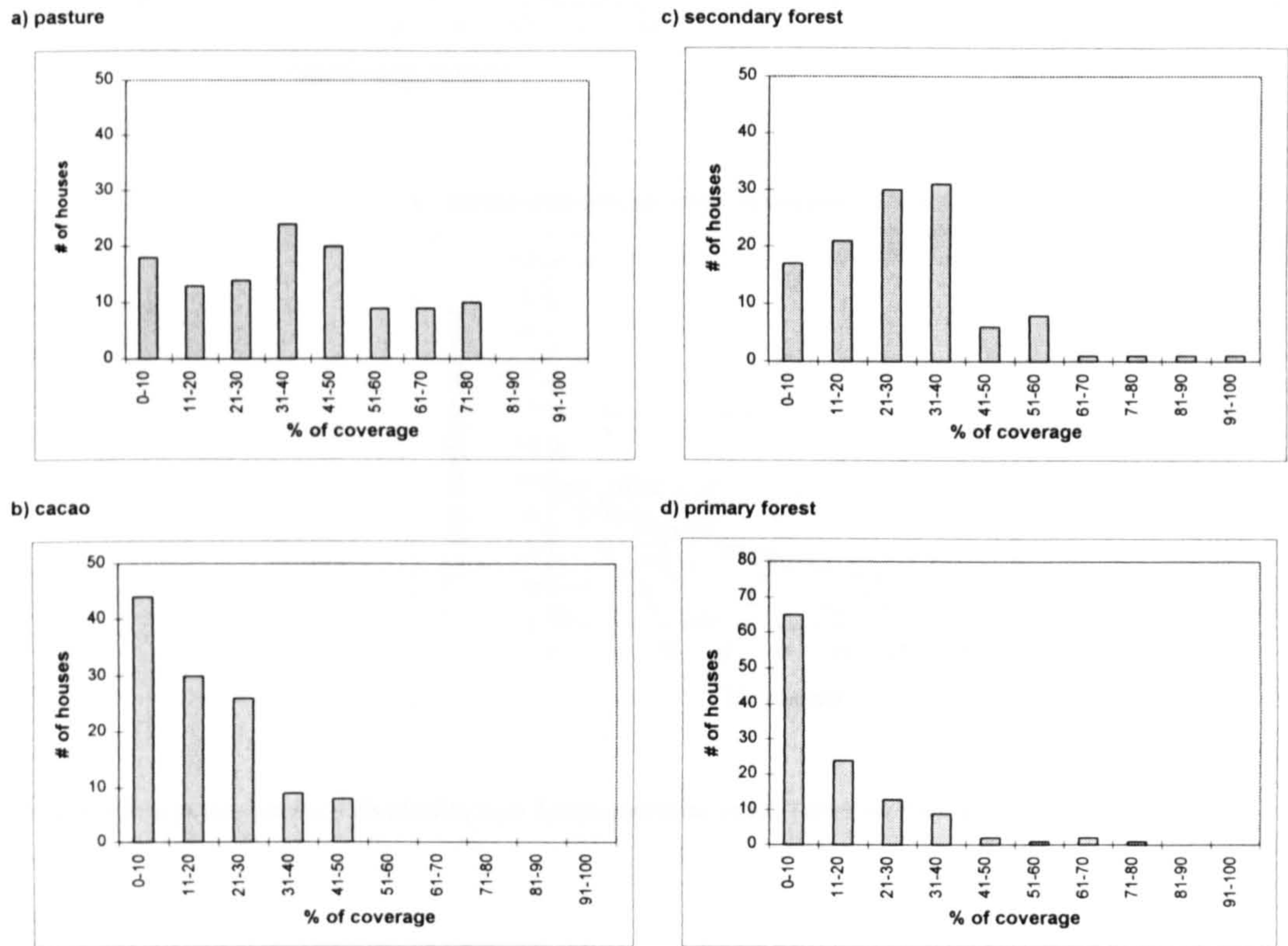
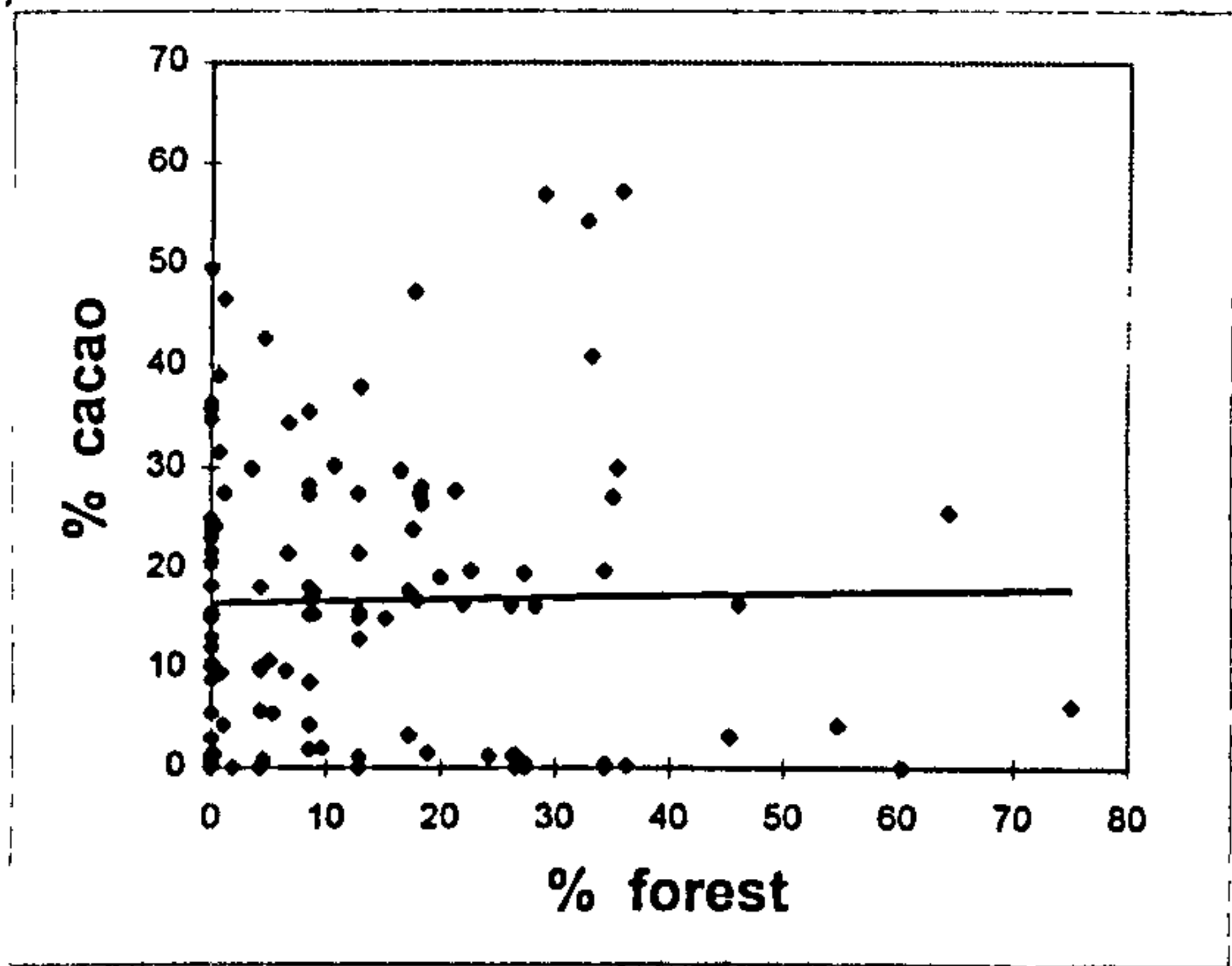
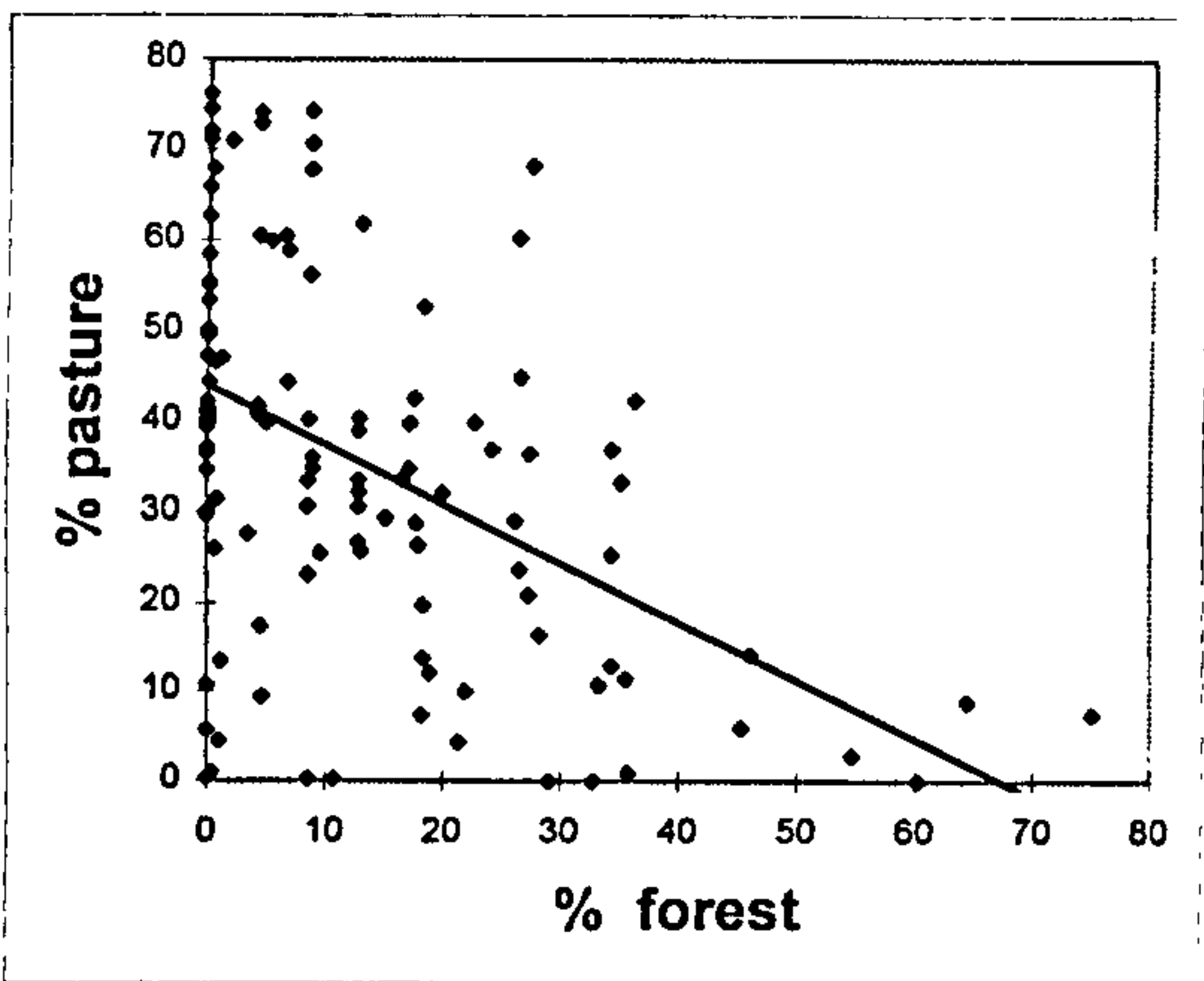


Figure 2.7 Relationship between vegetation features: land use

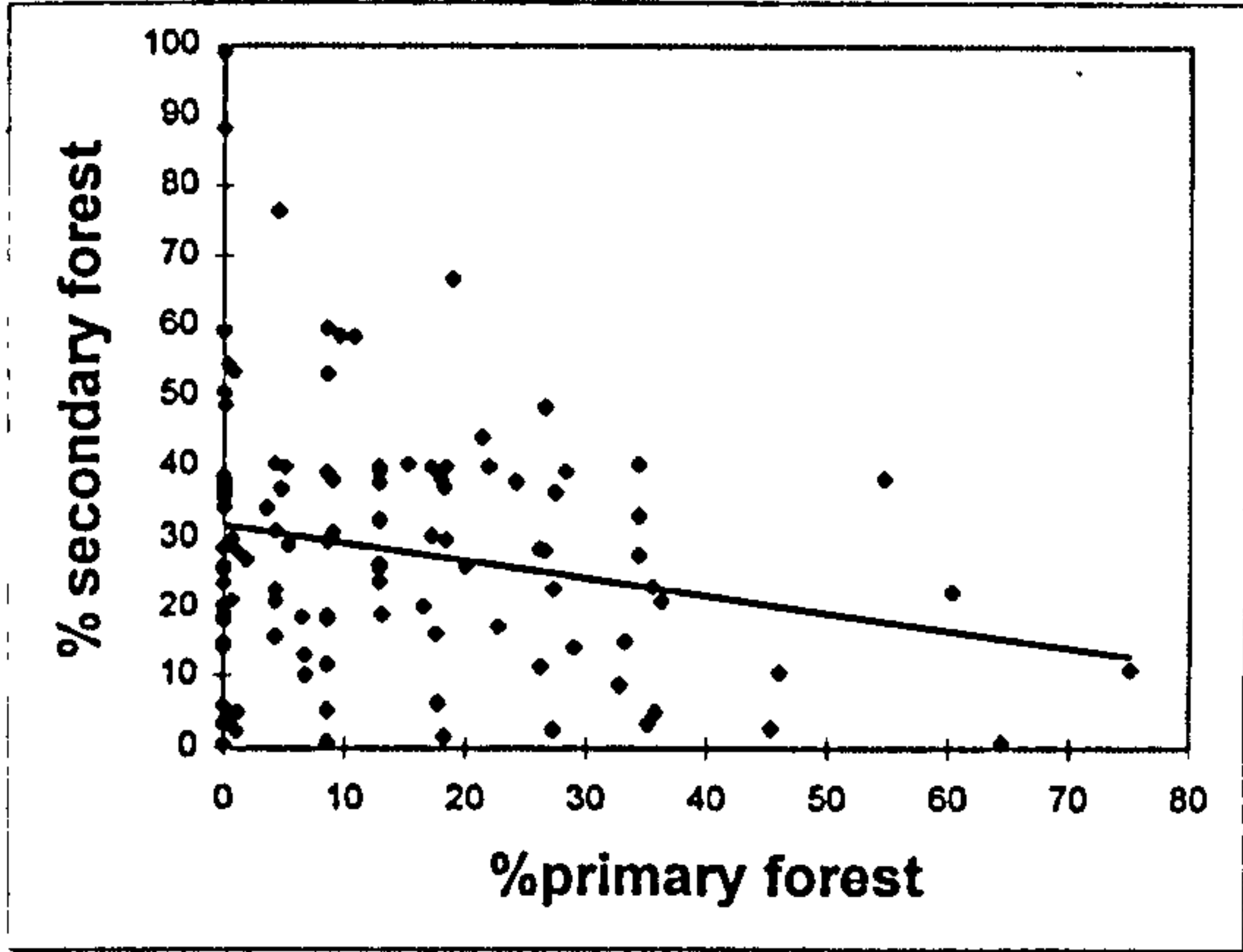
a) Relationship between cacao and primary forest



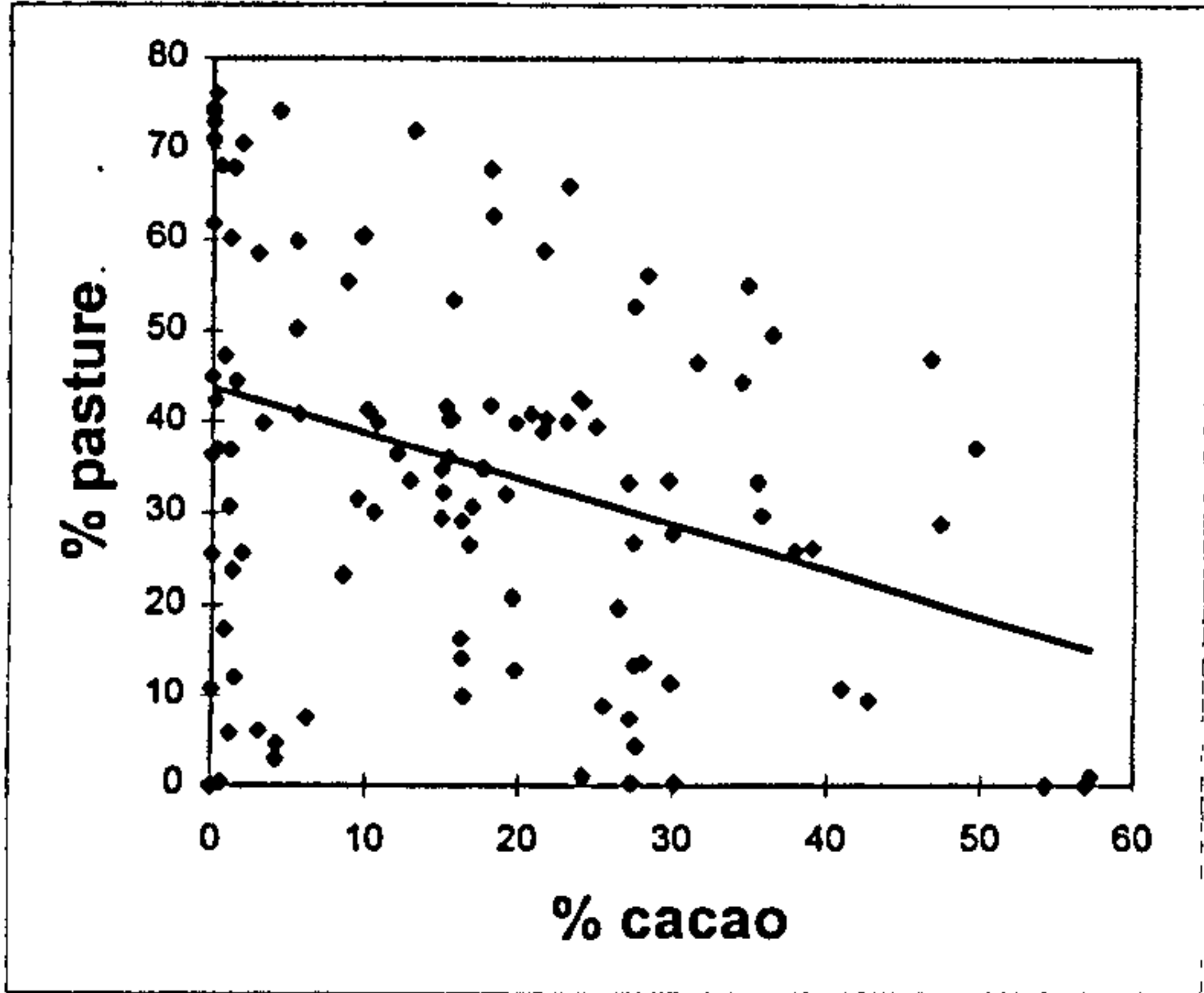
b) Relationship between primary forest and pasture



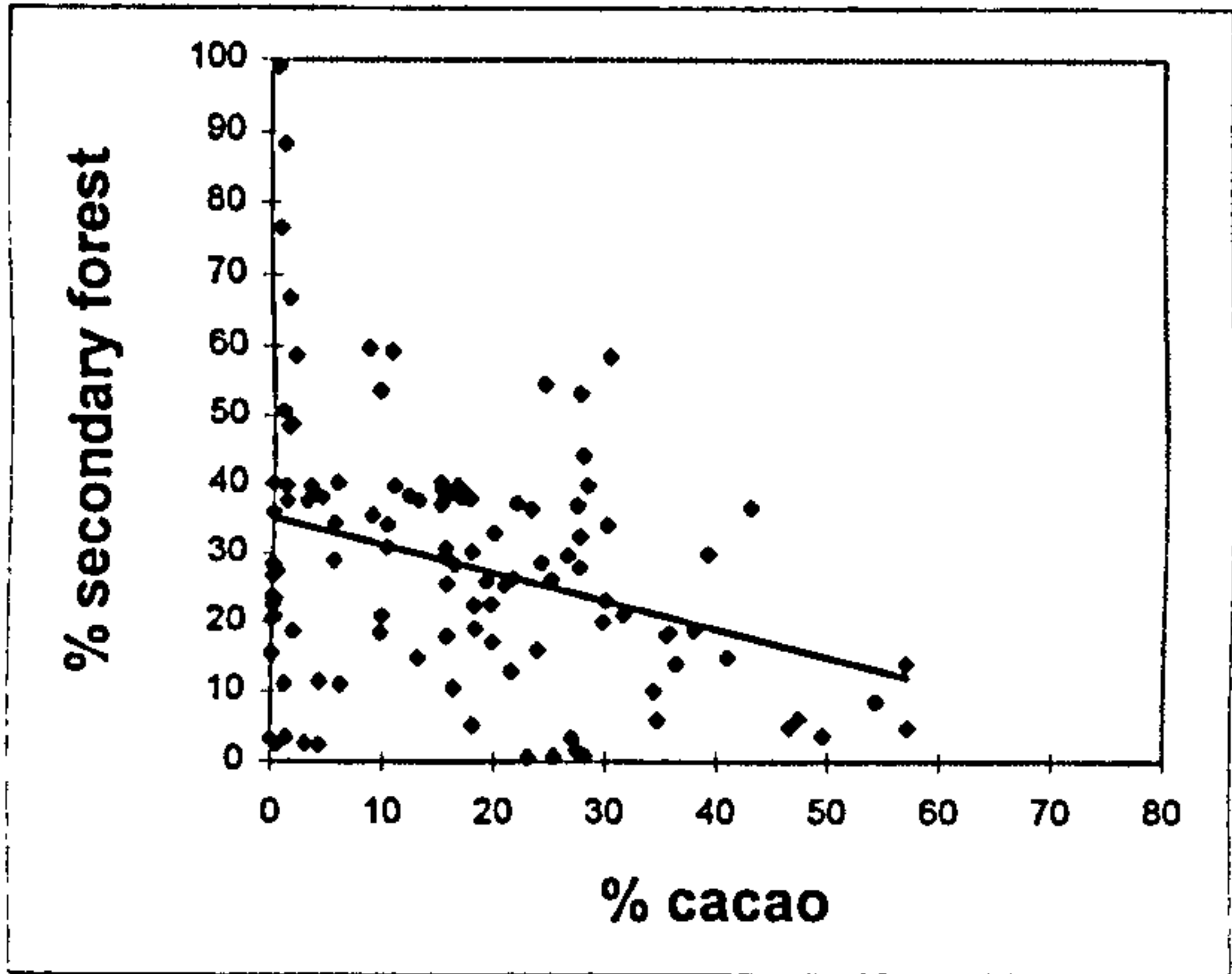
c) Relationship between primary and secondary forest



d) Relationship between cacao and pasture



e) Relationship between cacao and secondary forest



Points represent the data for individual houses. Lines represents trends (fitted by Excel)

Figure 2.8 Rainfall patterns in the Opon focus, 1996

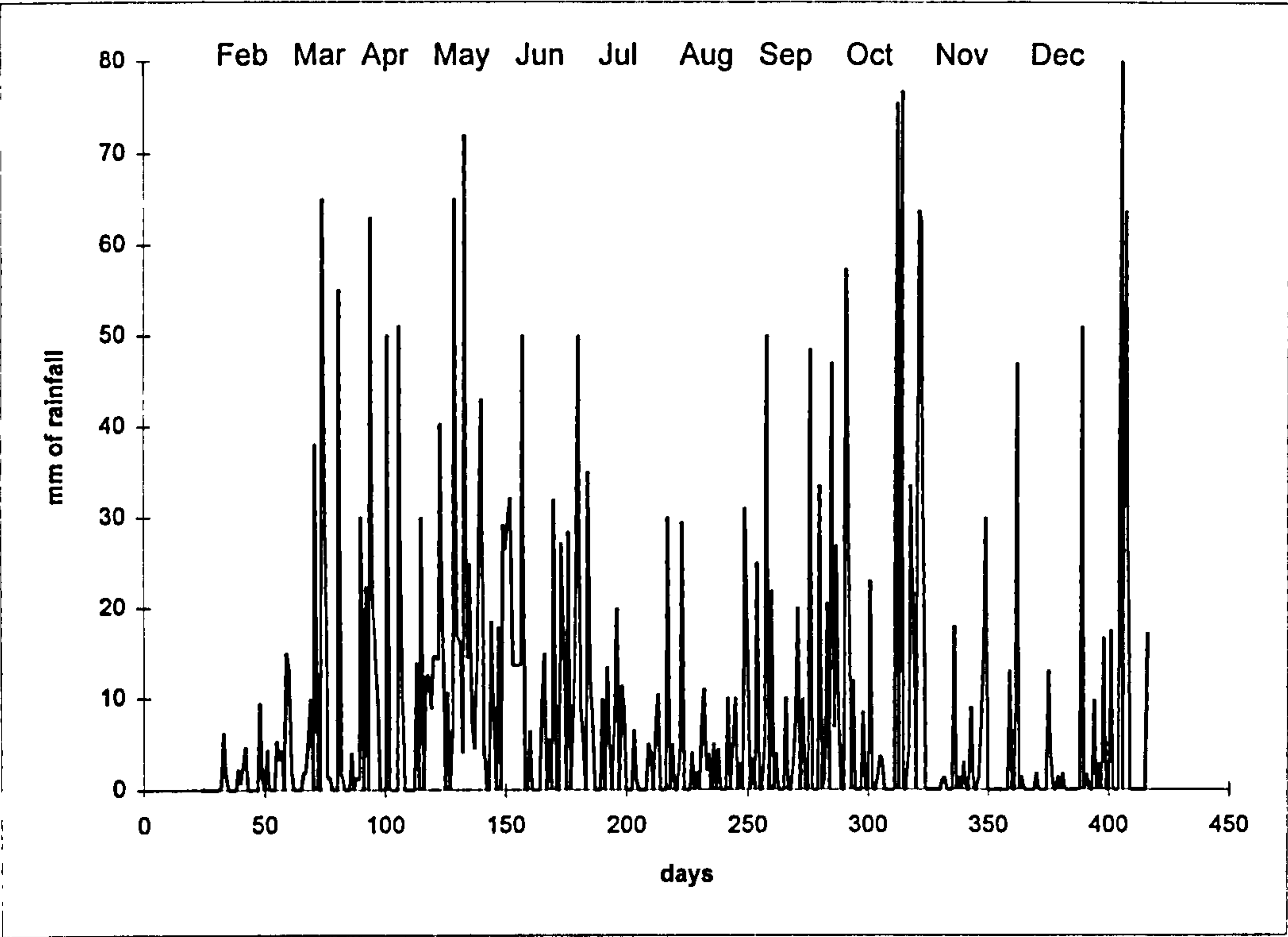


Figure 2.9 Frequency of rainfall in the Opon focus, 1996

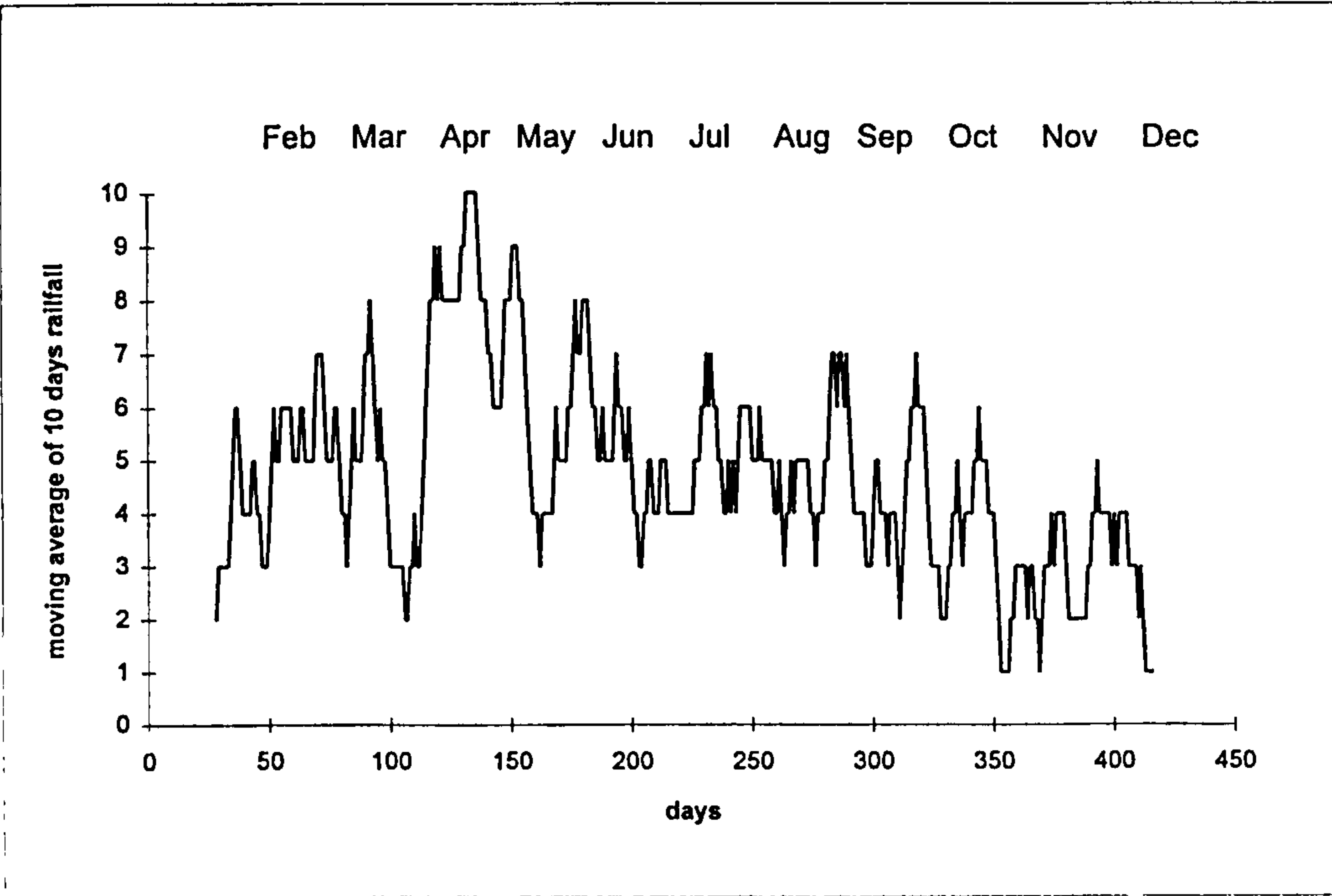


Figure 2.10 Minimum temperature pattern in the Opon focus, 1996

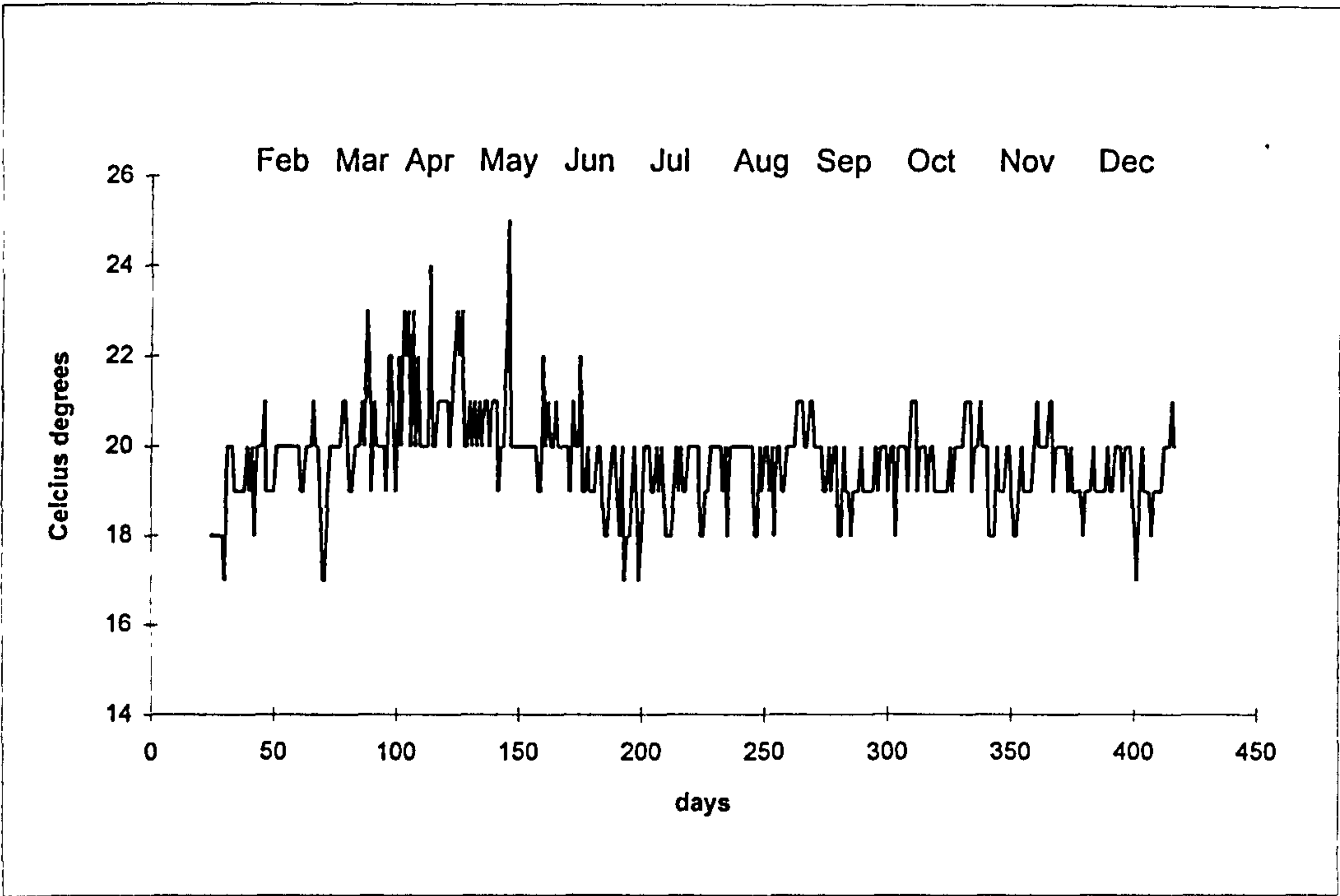


Figure 2.11 Maximum temperature pattern in the Opon focus, 1996

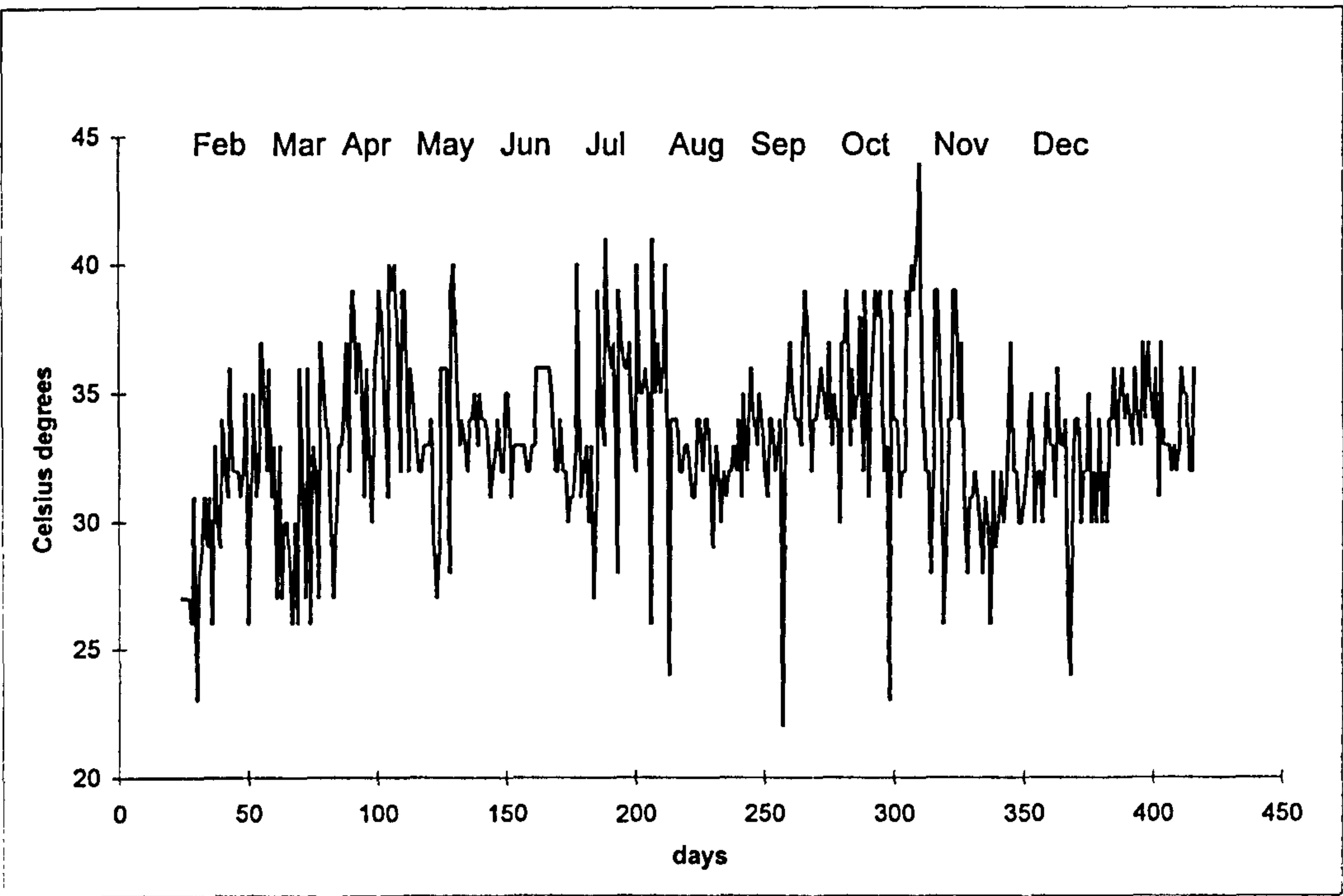


Figure 2.12 Relative humidity pattern in Opon focus, 1996

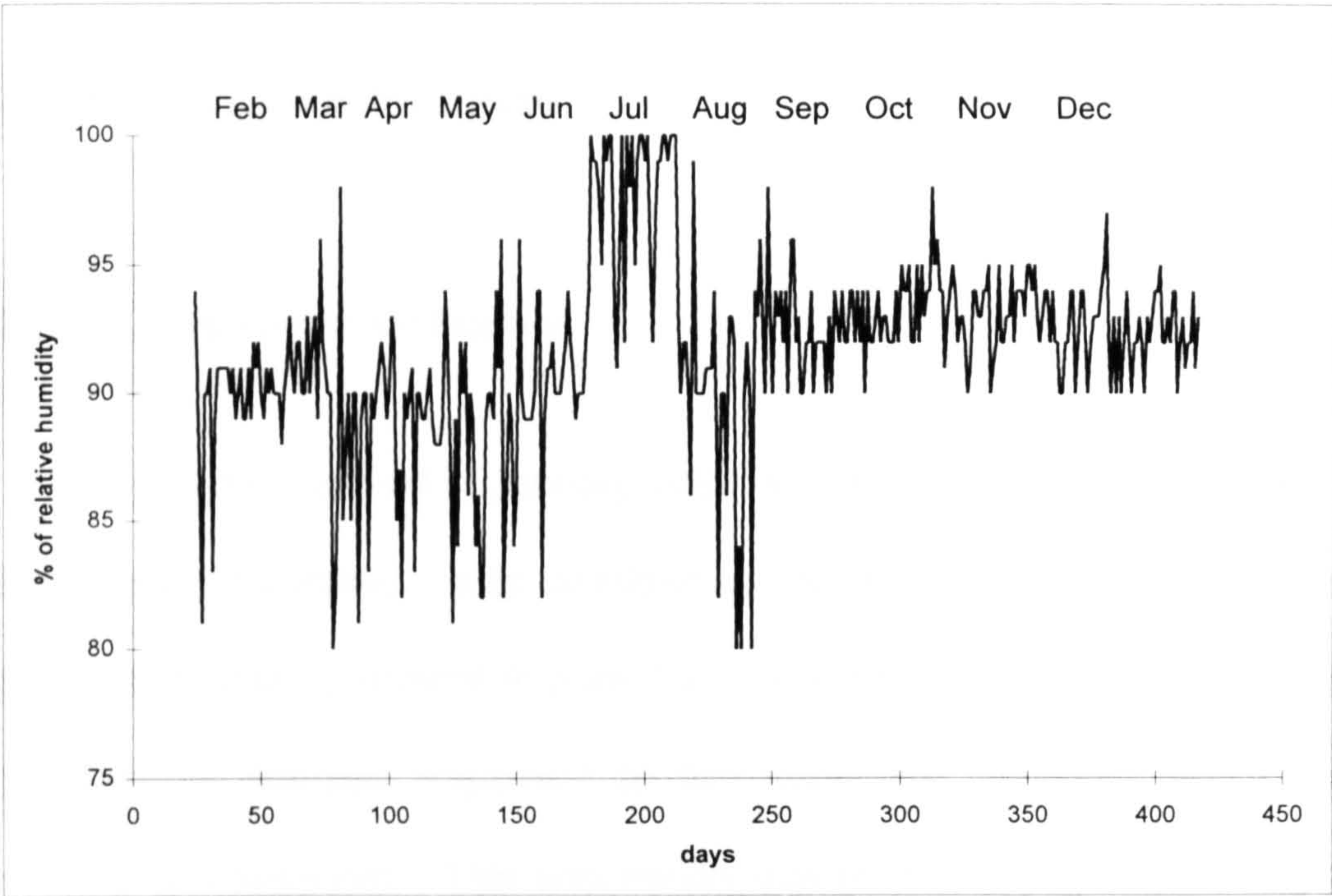


Table 2.1 Climatic conditions in the Opon focus, 1996

MONTHS	RAINFALL	TEMPERATURE		RELATIVE HUMIDITY	# DAYS WITH RAIN
		MINIMUM	MAXIMUM		
FEBRUARY	50.8	19.6	31.9	90.3	15
MARCH	207.2	19.9	31.6	89.6	16
APRIL	263.2	20.9	35	88.9	24
MAY	368.5	20.8	33.4	88.5	19
JUNE	231	20	33.3	91.7	18
JULY	102.8	18.9	35.2	97.9	15
AUGUST	82.9	19.6	32.2	89.5	14
SEPTEMBER	150.3	19.8	34	92.5	16
OCTOBER	275.3	19.3	34.6	93	13
NOVEMBER	273.7	19.8	34.2	93.6	10
DECEMBER	93.1	19.5	31.9	92.9	8
JANUARY	54.8	19.4	32.8	92.4	10

2.2 STUDY DESIGN

2.2.1 Human population study

2.2.1.1 Cross-sectional Survey

During the first visit in January 1995, a scaled map for each village was completed by modifying a map provided by the Agustin Codazzi Geographic Institute in Bogota, Colombia (Figure 2.2). The previous map did not contain information of features important to this study (i.e. houses, health post, churches, and markets). This was mainly due to restrictions imposed by the government in war areas: in Opon, a war between left wing guerrillas and the government army has been taking place for 30 years. Every house in the 12 villages was recorded and a code was assigned, consisting of the first two letters of each village name and a number (e.g. LS14 for house 14 located in La Soledad). The corresponding code was painted on the front wall of each house.

The map was used for planning and conducting the census, for MST application during the cross-sectional study, and for the follow-up of the cohort. During the census, a unique identification number was allocated to each person in every household. The number consisted of the house

number, followed by a continuous sequence starting with the household head as number one. Thus, BA1405 corresponds to the fifth eldest member of a family living in house 14 located in Buenos Aires village. The census used is shown in Appendix 1. The Colombian national identity card was requested at the time of the census in order to obtain the accurate date of birth. If possible, the whole family attended the interviews in order to avoid recall bias, when answering questions such as "age when infected".

The census was carried out for each house, recording information related to population structure (age, sex, and date of immigration, when appropriate) and clinical status (including age when infected). Past cases of leishmaniasis were identified by characteristic scars, which were detected by the author using the following criteria: no history of trauma, duration for > 2 weeks, central depressed surface and contours with no sharp angles.

Suspected leishmaniasis lesions were also defined by the absence of prior history of trauma and more than two weeks of evolution. A clinical history was made for each patient, including the time of disease onset, location on the patient's body, size, number, dermatological description and associated lymphatic involvement (if any) (Appendix 2). For mucous lesions, clinical diagnosis was taken into account: signs ranging from hyperaemia and infiltration associated with nasal problems, to ulcerations, perforations with much destruction of tissue and facial disfigurations. After clinical

inspection, the study population was classified into two groups: L+ (number of people with scar or lesions) and L- (number of people with no history of leishmaniasis). Lower case letters, l+ or l-, are used when referred to proportions of a particular sub-population belonging to L+ or L- respectively (Figure 2.14).

The cross-sectional study generated precise measurements of the prevalence of active lesions, scars and MST. In addition, the data provided retrospective estimates of incidence rates, both by fitting a simple model to the age prevalence data (see statistical analysis below), and by utilising the historical information on the dates of past infections.

2.2.1.2 Montenegro Skin Test (MST)

The Montenegro Skin Test (MST) followed the technique recommended by the World Health Organisation (WHO, 1990), i.e. using a mixture of *L. panamensis* and *L. braziliensis* at a concentration of $5 \times 10^6 \text{ ml}^{-1}$ heat-killed promastigotes for each species. 0.1 ml of leishmanin was injected intradermally on the external surface of the arm (Figure 2.13) using a pressurised intradermal injector (Dermo-jet model G, Robbins Instruments, USA). The injector produced results comparable to those obtained by intradermal test using syringes (Souza et al, 1992). The study population with MST was classified into two groups according to MST response, after the

diameter of induration was measured 48 hours later: MST negative (M-) when MST was less than 3 mm and MST positive (M+) when MST was equal or bigger than 3 mm. Lower case letters, m+ or m-, are used when referred to proportions of a particular sub-population belonging to M+ or M- respectively (Figure 2.14).

Figure 0.1 Site on the arm where MST was applied



1.1.1.1 Prospective longitudinal study

All households were visited five times at three months intervals following the initial cross-sectional survey, checking for changes in the study

population (e.g. due to immigration, emigration, birth and death) and looking for new LCL cases. During the final visit (February 1997) the study population was given a second MST using the same batch of antigen as before. The results provided precise estimates of incidence of infection according to age, sex, and clinical/immunological status at the start of the survey (Figure 2.14).

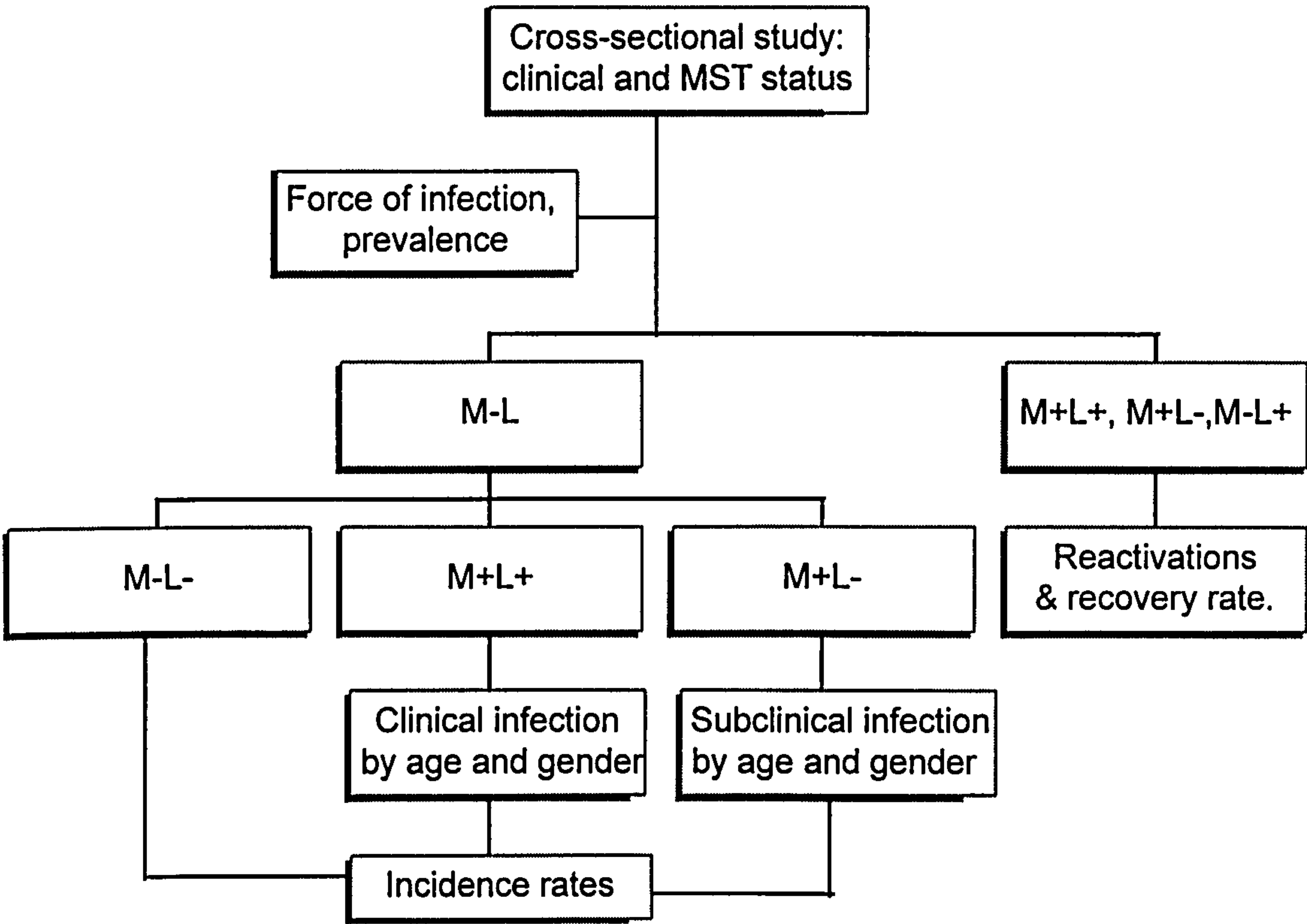
2.2.1.4 Measurement of transmission rates

The transmission rates were measured using the results of both the cross-sectional and longitudinal studies. The data analysis of the cross-sectional data focused on the comparison of prevalence rates (active or cumulative; MST responsiveness or clinical) according to sex and age. In addition, a simple infection-recovery model (Davies et al., 1995a) was fitted to the age prevalence curves (i.e. the proportion that were infected at age “a”) by maximum likelihood (Williams and Dye, 1994). The model assumes that susceptibles have become infected at a constant rate “ λ ” (the force of infection), and infecteds have recovered and returned to the susceptible class at a constant rate “ ρ ” (the recovery rate). It follows that the proportion infected at age “a” can be predicted from the equation:

$$p(a) = \frac{\lambda}{\lambda + \rho} (1 - e^{-(\lambda + \rho)a})$$

Incidence rates were also calculated from MST and/or clinical status conversion rates obtained from the prospective study (Figure 2.14). For comparative purposes, cohorts were defined by their initial MST and clinical status (as well as by age and gender), i.e. during the first cross-sectional survey.

Figure 2.14 Diagram of the epidemiological study



2.2.2 Sandfly Vectors

Sandflies were collected in Opon in order to provide complementary information for direct comparison with the temporal and spatial patterns of transmission. The sandfly study was divided into four parts: (1) endophagic activity in relation to the surrounding vegetation, (2) hourly (nocturnal) biting activity, (3) seasonal variation in the abundance of sandfly species during a year, and (4) natural *Leishmania* infections. Details of the number of person or trap/hours employed, sites and number of replicates for each part are given in Chapter 5. All results are expressed as the geometric mean number of sandflies (+1) collected.

2.2.2.1 Collecting Methods

CDC light traps were used to examine endophagy. These were battery-operated and they had previously been shown to provide useful and reliable collecting method under meteorological conditions similar to that in the Opon focus (Chaniotis et al, 1971b). Human landing catches were also used to examine nocturnal activity and seasonal variation. Four persons collected sandflies with aspirators simultaneously at the forest, cacao, peridomicile and intradomicile. People rotated between sites in order to minimise the effects of differences among workers (both in their attractiveness to the sandflies and in their collecting abilities). Collectors

were protected against sandfly bites by long sleeve shirts and long trousers; however they did not wear shoes. Human landing catches were also used to collect sandflies for the detection of natural infections.

2.2.2.2 Species Identification

Sandflies collected for endophagic activity, nocturnal activity, and seasonal variation studies were preserved in 70% ethanol and cleared in hot 10% KOH, followed by 100% phenol, whilst individuals for the natural infection study were cryopreserved in liquid nitrogen (Young et al, 1987b). Flies were identified to species according to Young and Duncan (1994), i.e. examining differences in the spermathecae, heads, cibaria, and hindlegs in females; and terminalia, genital pumps & filaments, and flagellomeres in males. Within the *Verrucarum* group, some females are similar in structure, and it is impossible to identify individuals in the absence of conspecific males (Kreutzer et al, 1990). Thus, the identification of *Lu. quasitowsendi* in Opon, which belongs to the *Verrucarum* group, required examination of the first generation males bred in the laboratory, from eggs laid from single *Verrucarum* group females.

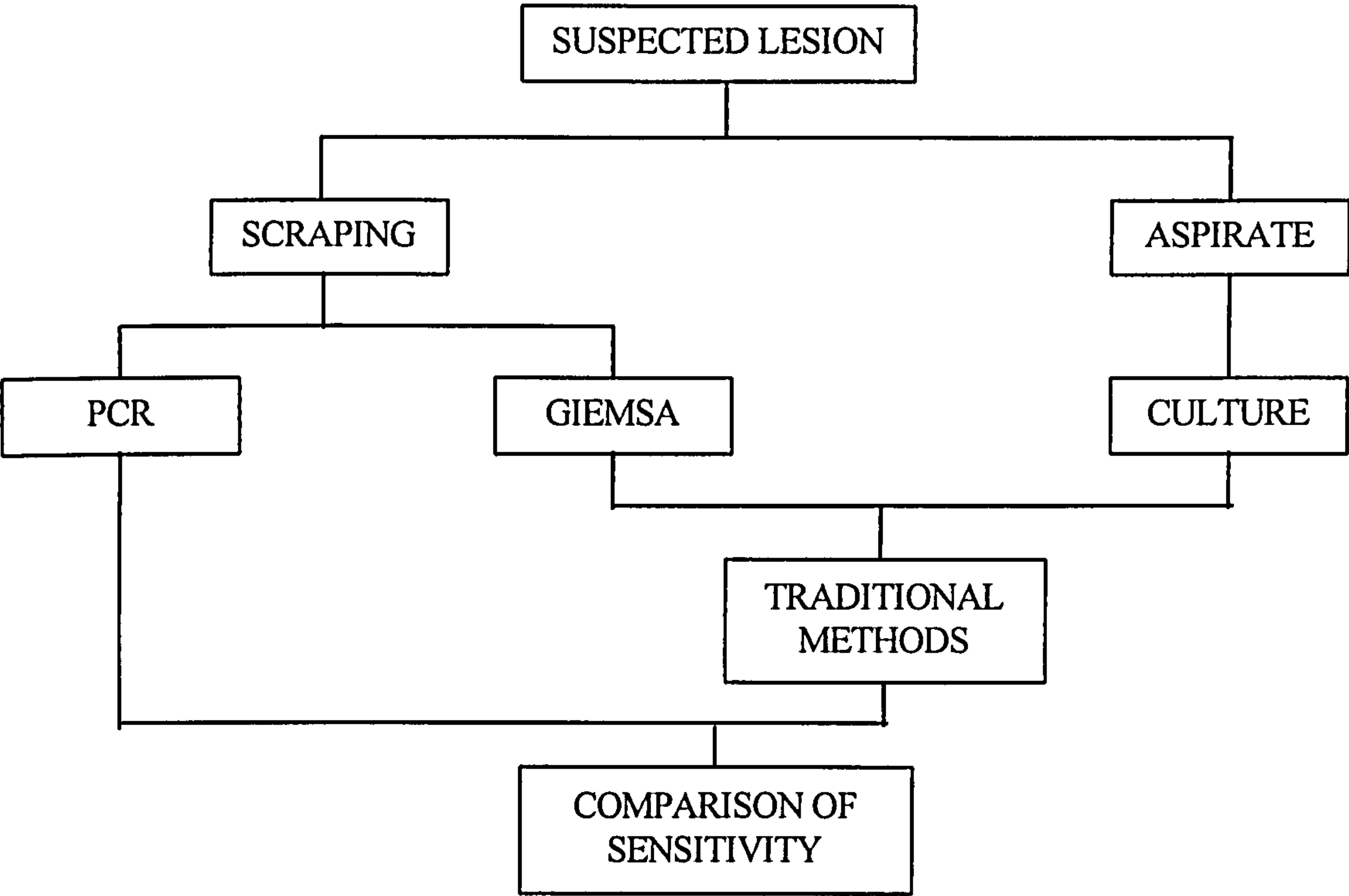
2.3 DIAGNOSTIC METHODS

In general, diagnostic procedures for cutaneous lesions included (i) microscopic examination of slides made from dermal scrapings, and (ii) the *in vitro* culture of parasites from aspirates (taken from the active edge of the lesion inoculated into biphasic media, Figure 2.15). In some patients, with persistent lesions, a biopsy was taken and fixed in 10% formalin. Most of the diagnostic procedures were made according to Weigle et al (1987) but with some modifications: (1) primary cultures were made using NNN biphasic medium: because past evaluations carried out in Santander Department demonstrated that NNN is more cost-efficient than Senekjje's medium (Ocazonez and Munoz, unpublished); (2) the dermal tissue was scraped using surgical blade number 15 with the sharp side facing out from the slit made in the outer border of skin lesion; and (3) the biopsy was only performed on patients with a clinical suspicion of another aetiology, rather than leishmaniasis: because, from past experience, bacterial contamination of the skin is a common problem amongst farmers in scattered dwellings.

Diagnosis of cutaneous lesions was also made by the Polymerase Chain Reaction (PCR) using dermal scrapings, air dried onto microscope slides, fixed in methanol, and kept at room temperature (Barker, personal communication, Figure 2.15). PCR procedure for mucous lesions used nasal scrapings which were kept onto NET10 SDS 1% buffer before DNA

extraction. All patients identified were provided with free treatment (Glucantime®) and followed-up on a monthly basis. Clinical histories and historical data were collected using “protocol 2” (see Appendix 2)..

Figure 2.15 Diagnostic methods used in Opon



For PCR, once in the lab. 50 µl of water was placed onto the slides for ca. 2 minutes and, following water lysis, the water lysate was transferred to an epindorf tube. This procedure requires no DNA extraction stage. The primers used were B1-extended universal([5'] GGG GTT GGT GTA ATA TAG TGG [3']) and B2-braziliensis specific([5']CTA GTG CAC GGG GAG G [3']) (deBrujin et al, 1992). 2 µl of sample lysate were amplified in 50 mM KCl, 10

mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, and 0.01% gelatin. 0.2 mM of each deoxyribonucleotide, 100 pmol of each primer and 2.5 units of Taq DNA polymerase (Perkin Elmer, UK) were used. Finally, the sample (50ul of reaction mixture and 2ul of water lysate) was overlaid with 100 µl of mineral oil (Sigma, UK), and denaturated at 96°C prior to a series of 35 cycles consisting of annealing (67.5 °C/1 min.), extension (72 °C/1 min) and denaturation (93 °C/0.5 min) and a final extension step of 10 minutes at 70 °C in order to ensure that all the PCR products were full length. Programmes were run on a 24-well Cambio Intelligent Heating Block (Genesys Instruments, Cambridge, UK).

2.4 PARASITE CHARACTERISATION : ISOENZYME ELECTROPHORESIS (IEA)

Eight enzyme systems were used for classification and discrimination of isolates from patients: Dipeptidase D (PEPD, E.C.3.4.13.9); nucleoside hydrolase (NH, E.C.3.2.2.1); mannose phosphate isomerase (MPI, E.C.5.3.1.8); malic enzyme (ME, E.C.1.1.1.40); glucose phosphate isomerase (GPI, E.C.5.3.1.9); phosphoglucomutase (PGM, E.C.2.7.5.1); 6-phosphogluconate dehydrogenase (6PGD, E.C.1.1.1.49); and esterase (ES, E.C.3.1.1.1). The enzyme extraction from cultured parasites was carried out according to Evans et al. (1989). 60 ml of logarithmic phase culture of parasites was collected and washed with PBS. The pellet was

treated with 2 mM stabiliser solution (2mM EDTA, 2 mM ϵ -aminocaproic acid, 2 mM dithiotreitol). The suspension was freeze-thawed three times in liquid nitrogen and then microfuged at high speed (12-15 Krpm) for 30 min at 4°C. The supernatant was collected and stored in liquid nitrogen until needed. Characterisation of *Leishmania* isolates was carried out by IEA in thin-layer starch-gel electrophoresis as described by Godfrey & Kilgour (1976) and Harris & Hopkinson (1976)

2.5 NATURAL INFECTION OF SANDFLIES

Sandflies were collected (for logistic reasons) at only two time-points, one in the dry and one in the wet season. Cryopreserved females were individually dissected in PBS under a compound microscope. First the head was cut off and then a small lateral incision was done in the last segments of the abdomen without tearing it. The end of the abdomen was pulled slowly away for extraction of the gut and transferred to other PBS drop and examined under light microscope in search of flagellates. Infected flies were placed onto a container with 50 μ l of PBS and macerated with dissection needles. 25 μ l of the macerate were placed in NET10 SDS1% buffer for DNA extraction and complementary PCR. The remaining 25 μ l was inoculated into a hamster nose. All dissections were carried out with fresh needles in order to avoid DNA contamination on PCR.

2.6 STATISTICAL ANALYSIS

Univariate or multivariate analysis were performed using general linearised modelling techniques in GLIM (v. 4.07) (Crawley, 1993). The estimates generated by these analyses are the coefficients of a linear model which is defined by the error structure, the linear predictor and the link function. In some cases, when non-normal errors were detected, the response variable was log transformed or a non parametric method was adopted. However, in general, when the outcome data were counts (e.g. the number of scars), Poisson errors were specified: and when the outcome data were proportions (e.g. prevalence), these were treated as binomial variables in logistic regression. For analyses of outcome data which were binary (e.g. healthy versus with disease) the data were put in as 0's or 1's, and the models assumed that the 0's and 1's came from a binomial trial with sample size 1 (i.e. specific binomial errors). The model chosen for each analysis is described in the result section.

2.6.1 Model simplification

Model simplification to the MAM was achieved by backward stepwise elimination. Each explanatory term was tested by its removal from the maximum model. The absolute or relative change in residual deviance

caused by its removal was examined for significance (see significance testing below); once all explanatory terms were tested, the insignificant parameters were then removed. Interaction terms were assessed and removed in order of diminishing complexity, i.e. before the main effect terms. This general procedure was repeated until the minimum adequate model, by definition, retained only explanatory variables which caused a significant increase in residual deviance when removed. When an interaction term was significant, its' main effects parameters were not removed from the model, except when of biological interest and appropriate, in which case, it was tested in the absence of its interaction with other variables.

2.6.2 Factor level simplification

The most parsimonious combination of factor levels was achieved by examining the change in residual deviance when replacing the original term with the test set of combined factor levels. An insignificant change in deviance indicated that the new grouping was appropriate.

2.6.3 Significance testing

For MAMs with binomial or poisson error structures, changes in residual deviance were compared to χ^2 distribution. In the event of overdispersion of

the MAM residuals (i.e. residual deviance \gg residual degrees of freedom), the scale parameter was transformed using the Pearson χ^2 , and the residual changes compared to the F, and not the χ^2 , distribution. The F-ratio statistic was also employed to test models with a normal error distribution. Significant deviation was defined as having a $\geq 95\%$ probability of not occurring by chance (i.e. $P \leq 0.05$). Finally, the fit of each model was checked by inspecting a plot of the residuals against the fitted values.

2.7 ETHICAL CONSIDERATIONS

In the Opon, the decision to participate in the cross-sectional and follow up studies was taken both at the community and household level. A verbal consent was received from each community leader (from each village) during a meeting held by the Landazury hospital which aimed to explain the whole project. Once in the area, each head of household was consulted by both the community leader and by the author. When the head of the household agreed to participate with the project, a delegation of the village leader and the author received the verbal consent. Written informed consent was obtained from all patients or, in the case of minors, from their parents or guardians. Everyone in the area surveyed received free medical care, regardless of whether they participated or not in the project. The study was approved by the Ethics Committee of the Industrial University of Santander.

3. DIAGNOSTIC AND CLINICAL FEATURES OF LEISHMANIASIS IN THE OPOF FOCUS

3.1 INTRODUCTION

The National Leishmaniasis Control Program (NLCP) in Colombia currently recommends the active search of leishmaniasis cases, as this strategy should enhance the likelihood of detecting early lesions, so improving the success of both diagnosis and treatment. Community involvement is essential for active search, diagnosis and treatment of leishmaniasis cases in distant foci. Thus, diagnostic tools suitable for this strategy requires the following criteria: (1) high sensitivity, (2) technical simplicity for sample collection, so that this can be carried out by a trained community member, (3) low lability of the samples (once collected) under field conditions, (4) and easily transportable. The NLCP also recommended that local hospitals should be the main source of Glucantime®, but there is not yet a clear policy for targeting high risk groups when drugs are in limited supply. In particular, a specific policy with respect to the treatment of persistently negative diagnostic results is required. This chapter describes the diagnostic and clinical features of the OpoF focus. It is hoped that the results will be

informative for NLCP, and will help the development of future policy recommendations. The specific objectives were as follows:

1. To evaluate the sensitivity and feasibility of diagnostic methods for tegumentary leishmaniasis under field conditions
2. To identify the *Leishmania* species responsible for leishmaniasis in Opon
3. To measure the variation in clinical response and to identify the risk factors for this variation

3.2 RESULTS

3.2.1 Diagnostic methods

Three diagnostic methods were carried out on a total of 168 patients: (1) 38 and 43 of whom were enrolled in the cross-sectional and follow-up studies respectively, plus (2) 87 patients who came from the excluded population or from neighbouring villages. The latter group (n=87) had a geometric mean age of 7 years (95% C.I. 5.7-8.3 years), and 52% (45/87) were male. The most common method used for diagnosis was the microscopic examination of Giemsa-stained slides made from dermal scrapings (n = 168), followed by *in vitro* isolation (i.e. culture) from aspirates (n = 141) and PCR (n = 49). Sixteen cultures (C) were bacterially contaminated before any *Leishmania* were seen: 15 were in a group tested by Giemsa (G) (with negative results) but not by PCR (P), and one was tested by Giemsa and PCR (with positive results by both methods). All contaminated cultures were treated as “not done” (ND) (Table 3.1).

The reliability of the results obtained from each method was tested by a series of comparisons where the result of a particular test was confronted with the results of the other test: (1) the percentage of positive PCR (%P+) was 87% in people with positive culture (Figure 3.1) (C+), which was significantly higher than %P+ for people with negative culture (C-) : 19% ($\chi^2 =$

16.22; 1 D.F.; $P < 0.001$); (2) the percentage of people with positive Giemsa (%G+) was 60% amongst C+, which was significantly higher than %G+ amongst C- : 31% ($\chi^2 = 10.12$; 1 D.F.; $P < 0.001$); and (3) amongst the C-group, %P+ was significantly higher amongst those who were G+ (60%) than amongst those that were G- (12%) ($\chi^2 = 3.59$; 1 D.F.; $P = 0.03$) (Table 3.2).

The true sensitivity of all three methods was not measurable because there is no “gold standard” for diagnosis. The following analyses therefore assume that all the clinically diagnosed patients were true leishmaniasis patients. Amongst the total population tested, there was no statistical difference between the percentage with a positive result by any of the three diagnostic tests (40%, 43%, and 44% by G, P and C respectively) (Table 3.3) ($\chi^2 = 0.38$; 2 D.F.; $P = 0.83$). When more than one diagnostic test was carried out on the same patient, the percentage of patients with at least one positive result increased (not surprisingly), with maximum sensitivity when both Giemsa and culture tests were combined (62%). Curiously, when all three methods were combined, the percentage with at least one positive result decreased to 50%. This decrease is due to selection bias in the use of a particular method. If one focuses only on those people who were tested by all three diagnostic methods ($n = 46$), then the sensitivity does increase as the results of more tests are incorporated, reaching a maximum sensitivity of 50% when all three methods are taken into consideration. Amongst this group of

46 patients, PCR was the most sensitive of the three diagnostic methods but not significantly different from the other two methods (Table 3.3).

Two sources of selection bias were identified for the application of the diagnostic tools in a particular patient: (1) culture attempts were more frequently made from patients with “typical” lesions: hence the %G+ was higher amongst this group than amongst those patients with no culture attempts; and (2) PCR was used for patients who were more likely to have negative results by either Giemsa or culture (Table 3.4). The strongest evidence for bias was detected amongst those patients with a negative culture: the %G+ was significantly higher amongst those who were not PCR tested (44%) than amongst those who were PCR tested (11%) ($\chi^2 = 11.64$; 2 D.F.; $P < 0.001$) (Table 3.4).

The best estimate of sensitivity for the results of combining more than one diagnostic method came from taking into account these biases in the selection of the sub-samples (Table 3.3). The expected sensitivity for each method, either individually or in combination, was calculated by using the best estimates of bias to predict how many of the untested group would be expected to have a positive result. For example, the expected sensitivity of combining PCR and Giemsa amongst the 168 patients is: $GP = [(P \cdot B \cdot N) + (R) + (G)] / 168$; where P is the proportion of people with negative Giemsa result who are PCR positive ($8/32 = 0.25$); B is the estimate of bias amongst the

Table 3.1 Distribution of leishmaniasis diagnostic test results by method

	GIEMSA (+)			GIEMSA (-)			TOTAL
	PCR (+)	PCR (-)	PCR ND	PCR (+)	PCR (-)	PCR ND.	subtotal
CULTURE (+)	9	1	23	4	1	17	55
CULTURE (-)	3	2	17	3	23	22	70
CULTURE ND	1	1	11	1	0	29	43
subtotal	13	4	51	8	24	68	
total	68			100			168

Table 3.2 Concordance of diagnostic methods

	all suspected patients				Giemsa +		Giemsa -	
	% PCR (+)		% GIEMSA (+)		% PCR (+)		% PCR (+)	
CULTURE (+)	13/15	87%	33/55	60%	9/10	90%	4/5	80%
CULTURE (-)	6/31	19%	22/70	31%	3/5	60%	3/26	12%
CULTURE ND	2/3	67%	13/43	30%	1/2	50%	1/1	100%

Table 3.3 Sensitivity of diagnostic methods

methods	all 3 methods tested		all suspected pat.		expected sensitivity	
GIEMSA (G)	15/46	33%	68/168	40%	68/168	40%
PCR (P)	19/46	41%	21/49	43%	84/168	50%
CULTURE (C)	15/46	33%	55/125	44%	68/168	40%
G - P	22/46	48%	25/49	51%	96/168	57.10%
G - C	20/46	43%	77/125	62%	96/168	57.10%
P - C	21/46	46%	21/46	46%	86/168	51.20%
G - P - C	23/46	50%	23/46	50%	104/168	61.90%

Table 3.4 Measurement of selection bias in the application of diagnostic test

population	diagnost.	percentage		relat.risk	C.I.	X ²	P
PCR done: all	%G (+)	17/49	35%	1.24	0.67 -	0.65	0.41
PCR ND: all	%G(+)	51/119	43%		3.02		
cult done: all	%G (+)	55/125	44%	0.69	0.24 -	1.98	0.15
cult ND: all	%G(+)	13/43	30%		1.22		
PCR done: all	%C (+)	15/46	33%	1.55	0.93 -	3.14	0.07
PCR ND: all	%C(+)	40/79	51%		4.9		
PCR done: C-	%G(+)	5/46	11%	4.01	1.87 -	11.64	< 0.001
PCR ND: C-	%G(+)	17/39	44%		24.5		

were not tested by PCR (100); R is the number of people who are Giemsa negative and PCR positive (8); and G is the total number of people with a positive Giemsa result (68). Thus, the expected sensitivity for a group of samples tested by both Giemsa and PCR is 57.1% (96/168) (Table 3.3). Using an analogous calculation, the best estimate of sensitivity when all three methods are combined is 61.9%.

3.2.2 Parasite classification

In total, 55 strains were isolated from 141 patients. Twenty five were typed by isoenzyme electrophoresis (8 isoenzymes) at the LSHTM, 15 were classified by monoclonal antibodies at the National Health Institute in Bogota, Colombia (all *L. panamensis*) and 10 were not classified due to heavy fungal contamination. All isolates tested by isoenzyme electrophoresis were identified as *L. panamensis* and 23/25 presented the same zymodeme pattern as the control strain used (reference code: MHOM/PA/71/LS94). The two exceptions were both isolates from geographic region 1 (Plan de Armas and Buenos Aires), where a single variant of PEPD was detected (samples 3 and 5 in Figure 3.2).

Figure 3.1 Positive results of PCR in samples from LCL patients

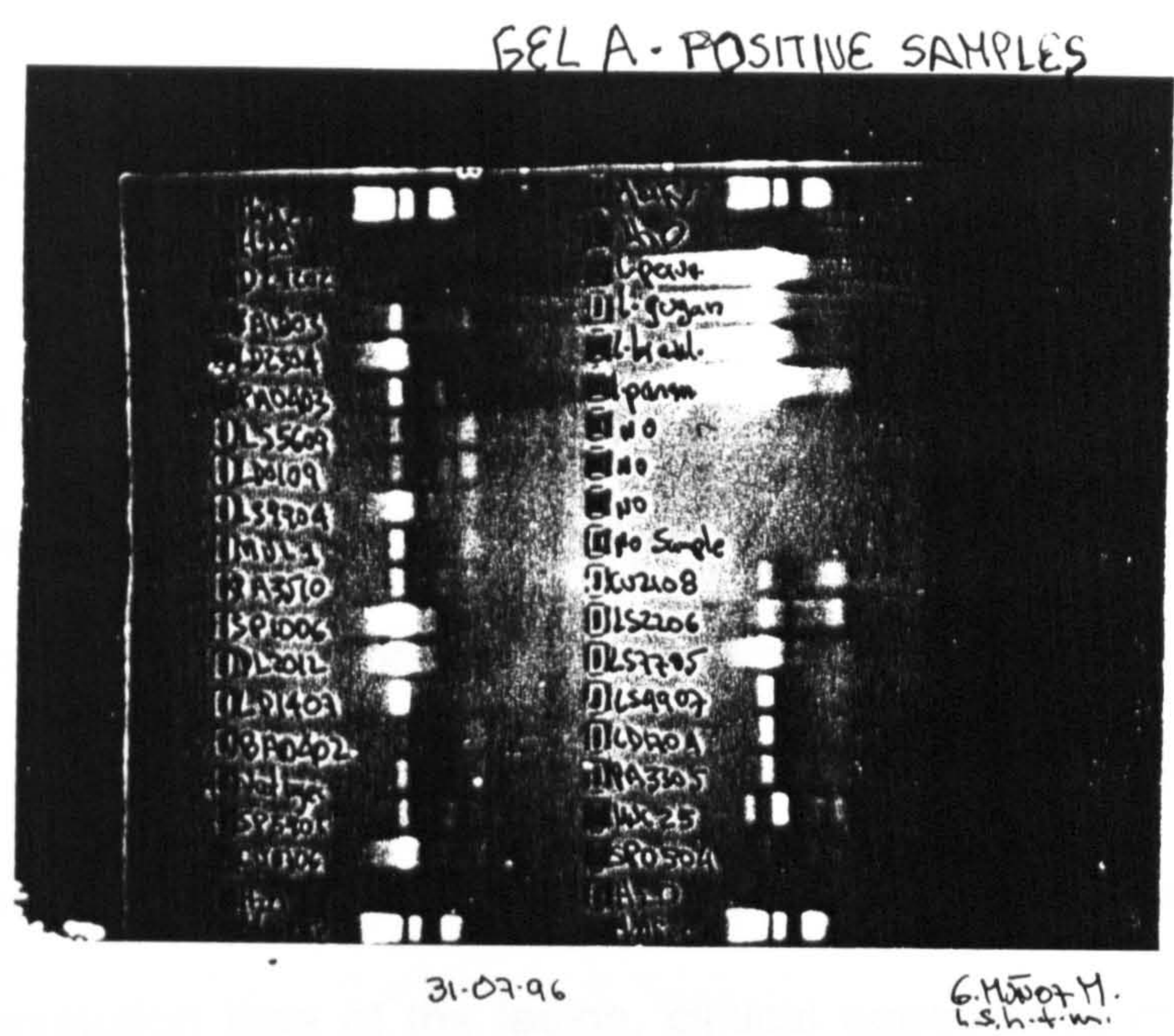
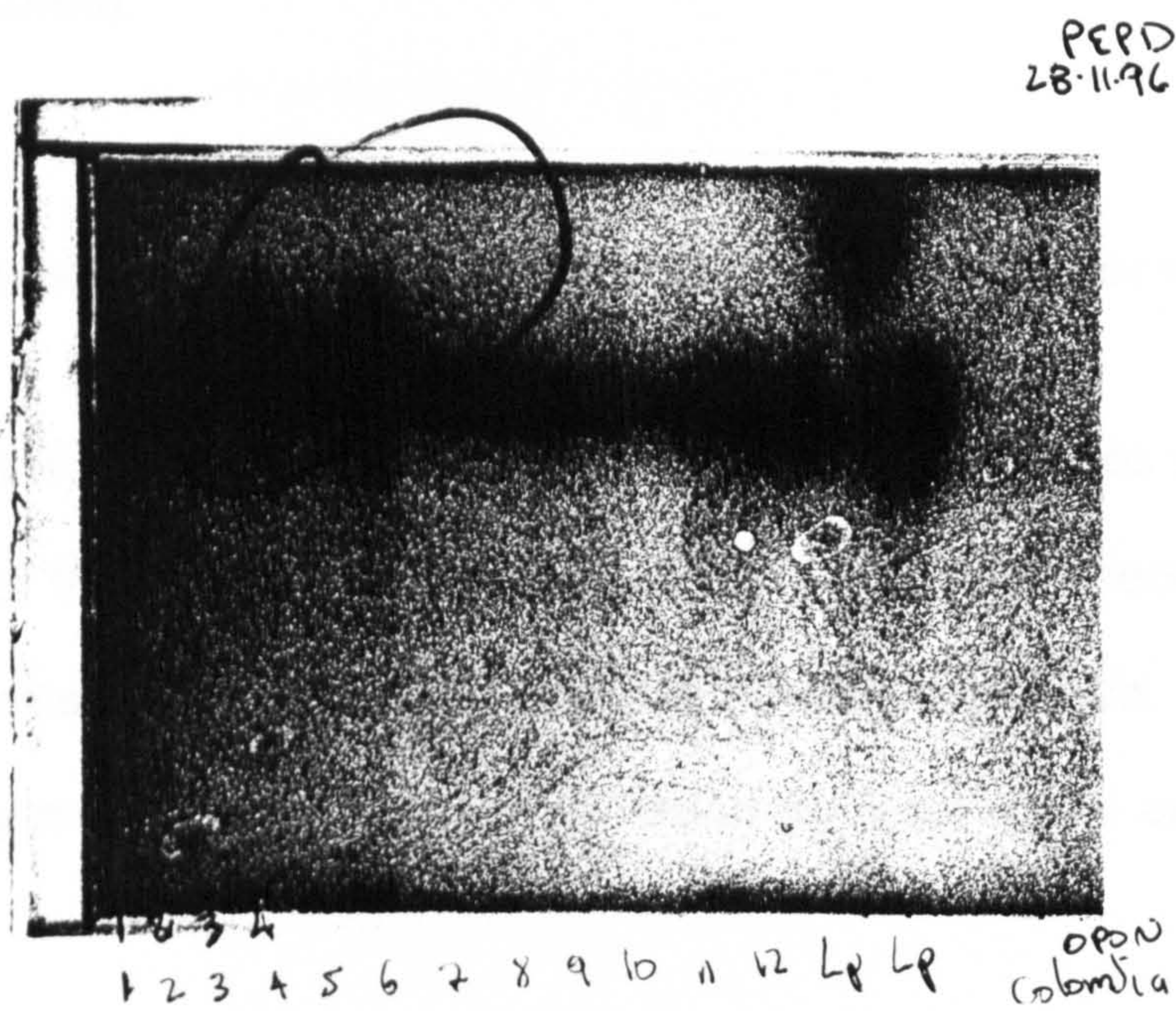


Figure 3.2 PEPD patterns in parasites from Opon (IEA)



3.2.3 Factors determining the size, number and site on the body of lesions or scars

Risk factors that determined the area, number and site of active or past leishmaniasis infections were sought both amongst LCL patients ($n = 168$) and amongst the scarred population ($n = 1187$). Both univariate and multivariate analyses were carried out in order to test for associations with the following factors: (1) the people's provenance (region), (2) personal factors (i.e. age at the time of infection, gender), and (3) area, number and localisation of lesions/scars. For the LCL patients, additional factors tested included: evolution time of the lesion, clinical appearance of the lesion (i.e. type, description, and presence of secondary infection or adenopathy) and presence of scars from previous infections. For the scarred population, a single additional factor was tested: "age now" (i.e. at the time when the scar was measured).

3.2.3.1 Risk factors associated with the size of lesions or scars

At the time when the census data and clinical histories were collected, the area of the largest lesion or scar on each patient was recorded. For all LCL patients, and for 454/1187 of the scarred population, this area was accurately measured by drawing the lesion (or scar) shape onto transparent

plastic paper. However due to logistic constraints, for 723 of the scarred population, the area of the largest scar was estimated from its length and width and assuming an oval shape. In order to standardise the two methods of measuring size, an equation was constructed by logistic regression in order to predict actual size from transformed "oval area" estimated for the 723 patients: $\text{actual size} = e^{[(0.732) + ((0.6122 \times \log(\text{oval-area}))]}$. Hence, all the following analyses are carried out on actual lesion/scar size (whether observed or estimated), following log transformation to generate a normal distribution.

a) Size of lesion

The overall geometric mean area of the largest active lesion on each patient was 1.52 cm², ranging from 0.13 to 95.4 cm² (n = 168). The univariate analyses demonstrated that lesion size differed significantly with respect to geographic region, localisation on the body, description and presence/absence of secondary infection or adenopathy (Table 3.5): (1) Lesions were significantly bigger in patients from region 1 (i.e. Buenos Aires, Cucuchonal and Plan de Armas) than in patients from region 3 (La Soledad), (2) lesions located on the head were significantly smaller than lesions on the legs, (3) verrucose lesions were significantly bigger than flat lesions, and (4) lesions with secondary bacterial infection were bigger than those without. The mean lesion area for male patients was 1.3 times bigger than for females, but the difference had only borderline significance.

Table 3.5 Risk factors associated with area and number of active lesions (univariate analyses)

OUTCOME VARIABLES		AREA OF LCL					NUMBER OF LCL				
EXPLANATORY VARIABLES FACTORS		n	geometric mean	observed lower limit	observed upper limit	univariate statistics	n	geometric mean	observed lower limit	observed upper limit	univariate statistics
REGION	1	43	2.3	0.3	26.9	F = 3.705 P = 0.0033 D.F. = 5,163	43	1.4	1	5	F = 1.69 P = 0.16 D.F = 5,162
	2	37	1.4	0.2	11		37	2.1	1	10	
	3	36	0.8	0.1	12.6		36	1.6	1	6	
	4	8	1.6	0.4	7.9		8	2.4	1	6	
	5	29	1.9	0.2	95.5		29	2.1	1	13	
	6	15	1.4	0.2	7.4		15	1.7	1	6	
GENDER	FEMALE	92	1.3	0.1	26.9	t = 1.91 P = 0.05 D.F. = 1,166	92	1.8	1	13	t = 0.72 P = 0.46 D.F. = 1,166
	MALE	76	1.8	0.2	95.5		76	1.7	1	6	
TYPE OF LESION	PAPULE	28	1	0.2	14.1	F = 2.16 P = 0.075 D.F. = 4,164	23	1.3	1	4	F = 0.129 P = 0.94 D.F. = 3,107
	NODULE	32	1.2	0.2	95.5		28	1.3	1	5	
	ULCER	64	1.9	0.1	26.9		47	1.3	1	8	
	MULTIPLE TYPE	29	1.9	0.2	15.8		exclud				
	BUBONIC	15	1.5	0.2	8.1		13	1.4	1	4	
LOCALISATION	HEAD	11	0.7	0.2	13.2	F = 3.46 P = 0.009 D.F. = 4,164	11	1.1	1	3	F = 2.58 P = 0.055 D.F. = 3,107
	ARMS	57	1.3	0.2	19.5		53	1.2	1	4	
	LEGS	48	2.1	0.1	95.5		41	1.5	1	8	
	TRUNK	6	1.3	0.2	3.4		6	1.7	1	3	
	MULTIPLE SITES	46	1.7	0.3	15.8		exclud				
PREVIOUS SCAR	NO	144	1.6	0.1	95.5	F = 2.12 P = 0.14 D.F. = 1,167	144	1.8	1	13	t = 0.71 P = 0.478 D.F. = 1,166
	YES	24	1.1	0.4	7.1		24	1.7	1	6	
DESCRIP. OF THE ULCER	FLAT	116	1.2	0.1	95.5	F = 8.59 P = 0.0002 D.F. = 2,166	116	1.7	1	13	F = 0.484 P = 0.62 D.F. = 2,108
	RAISED	47	2.3	0.7	26.9		47	2.1	1	8	
	VERRUCOSE	5	4.9	3	8.3		5	1.9	1	3	
OTHER	NONE	111	1.3	0.1	95.5	F = 3.73 P = 0.025 D.F. = 2,166	111	1.6	1	10	F = 0.607 P = 0.55 D.F. = 2,108
	SECONDARY BACTERIAL INF.	27	2.2	0.3	17.8		27	1.8	1	6	
	ADENOPATHY	30	2	0.4	11.7		30	2.3	1	13	
CONTINUOUS VARIABLES											
AGE	<6	71	1.5	0.2	26.9	F = 0.24 P > 0.05 D.F = 1,166	71	1.8	1	6	F = 0.82 P > 0.05 D.F. = 1,166
	6-15	52	1.5	0.1	17.8		52	1.6	1	10	
	>15	45	1.6	0.2	95.5		45	2.1	1	13	
EVOLUTION TIME (months)	1-5	127	1.6	0.1	95.5	F = 0.06 P > 0.05 D.F = 1,166	127	1.9	1	13	F = 1.59 P > 0.05 D.F. = 1,166
	6-12	26	1.4	0.2	19.5		26	1.8	1	6	
	>12	15	1.2	0.2	8.1		15	1.5	1	6	
AREA	0.13-3	125					125	1.9	1	13	F = 0.92 P > 0.05 D.F. = 1,166
	>3	43					43	1.6	1	6	
NUMBER OF LESIONS	1-3	138	1.5	0.1	95.5	F = 0.10 P > 0.05 D.F = 1,166	138				
	>3	30	1.5	0.2	15.8		30				
significant variables											

In contrast, a minimal adequate model with only one explanatory variable (the lesion's description) was obtained from the multivariate analysis by backward elimination from a maximal model with 11 clinical and personal variables. Lesions with raised borders had a mean area 4.5 times (95% C.I. 2.08-9.94) the mean area of lesions with flat borders; and the mean area of verrucose lesions was 5.7 times (95% C.I. 0.6 - 54.6) the mean area of lesion with raised borders ($F = 9.7$; $P < 0.01$; D.F. 2, 165). This model explains only 10.5% of the variance in lesion area (Table 3.8).

b) Size of scars

The overall geometric mean area of the largest scar on each healed patient was 2 cm², ranging from 0.3 cm² to 16.8 cm² (n = 1187). As for active lesions, the univariate analyses demonstrated that scar size differed significantly with respect to gender and localisation on the body; in addition, scar size differed significantly according to the age of the patient at the moment of the census ("age now"), and according to the number of scars (Table 3.6): (1) Scars were significantly bigger in people over 15 years of age (at the time when the scar was measured) than in younger people; (2) in contrast to the results for active lesions, scars were bigger in females than in males; (3) scars on people with at least four scars tended to be bigger than those on people with fewer; and (4) as for active lesions, scars located on the legs were significantly bigger than scars on other parts of the body.

Table 3.6 Risk factors associated with area and number of scars (univariate analyses)

OUTCOME VARIABLES			AREA OF SCARS						NUMBER OF SCARS					
EXPLANATORY VARIABLES FACTORS			n	geometric mean	observed lower limit	observed upper limit	univariate statistics	n	geometric mean	observed lower limit	observed upper limit	univariate statistics		
REGION	1		248	2.13	0.95	16.8	F = 1.865 P = 0.097 D.F. = 5	248	2	1	17	F = 0.48 P = 0.79 D.F. = 5,1181		
	2		212	2.04	0.6	15		212	2.2	1	18			
	3		191	1.98	0.5	15.3		191	2.2	1	30			
	4		191	1.88	0.3	14.75		191	2	1	15			
	5		209	2.03	0.7	15.9		209	2	1	27			
	6		136	1.96	0.3	8.3		136	2.1	1	27			
GENDER	FEMALE		633	2.05	0.3	16.8	t = 2.098 P = 0.036 DF. 1,1186	633	2.3	1	30	t = 3.38 P = 0.000729 D.F. 1,1185		
	MALE		554	1.95	0.5	15.9		554	1.9	1	17			
LOCALIS.	MULTIPLE LOCAL. ARMS LEGS HEAD TRUNK		390	2.22	0.3	16.8	F = 49.13 P < 0.0001 D.F. = 4	exclud				F = 5.5 P = 0.0013 D.F. = 3, 793		
			303	1.79	0.3	8.33		303	1.5	1	14			
			323	2.33	0.5	15.9		323	1.8	1	17			
			115	1.39	0.3	10		115	1.3	1	7			
			56	1.54	0.3	12.5		56	1.7	1	20			
CONTINUOUS VARIABLES														
AGE NOW	<6		88	1.59	0.3	11	F = 6.25 P > 0.05 D.F. =1,1185	88	2.3	1	17	F = 18.09 P < 0.05 D.F.1,1185		
	6-15		415	1.94	0.3	15.9		415	2.3	1	30			
	>15		684	2.11	0.3	16.8		684	1.9	1	27			
AGE OF INFECT.	<5		378	1.95	0.3	12.5	F = 0.39 P > 0.05 D.F. =1,1185	378	2.4	1	30	F = 17.61 P < 0.05 D.F.1,1185		
	6-15		438	2.08	0.3	16.8		438	2	1	17			
	>15		371	1.99	0.3	8.3		371	1.9	1	27			
AREA	<4		1143					1143	1.8	1	20	t = 3.97 P = 0.0002 D.F.1,1185		
	>3		44					44	2.4	1	30			
NUMBER OF SCARS	1-3		893	1.92	0.3	12.5	F = 62.27 P < 0.001 D.F.=1,1185							
	>3		294	2.29	0.5	16.8								

significant variables

In the MAM generated by multivariate analysis scar size was associated with number of scars, “age now”, age when infected, and localisation on the body. The following examples illustrate the predicted change in scar size associated with changes in each of these parameters. Using the population mean values for “age now” and age when infected, for people with single scars, the predicted mean scar size on the legs is 15 cm² compared to only 9 cm² on the rest of the body. For people with five scars, the size of the largest scar tends to be bigger (not surprisingly): 17 cm² on the leg and 11 cm² on other body parts.

We can illustrate the effect of age by focusing on people with single scars on the legs. A change in age when infected from 2 years to 13.8 years (the population mean) is associated with a decrease in scar size from 15 to 13 cm². In contrast, a change in “age now” from 14 to 50 years is associated with an increase in scar size from 15 to 20 cm². In summary, maximum scar size is predicted to increase with the number of scars and with “age now” but decreases with age when infected. The largest scars tend to be on the legs. This model explains 18% of the variance in scar size (Table 3.8).

3.2.3.2 Risk factors associated with the number of lesions or scars

Two factors used in the previous analyses have a direct and trivial positive relationship with the number of lesions or scar: multiple lesion types

and multiple sites are both obviously only possible for people with multiple lesions or scars. Therefore, patients with lesions ($n = 29$) or scars ($n = 390$) on multiple sites and patients with multiple lesion types ($n = 46$) were excluded, respectively, from the univariate analyses of the effects of localisation or lesion type on the number of lesions/scars. Then, in the multivariate analysis, both these factors were excluded from the maximal model, so that the whole study population could be included in the analysis involving the remaining factors.

a) Number of LCL

The overall geometric mean number of lesions was 1.8, ranging from 1 to 13 ($n = 168$). None of the variables tested were significant either in the univariate or multivariate analyses (Table 3.5).

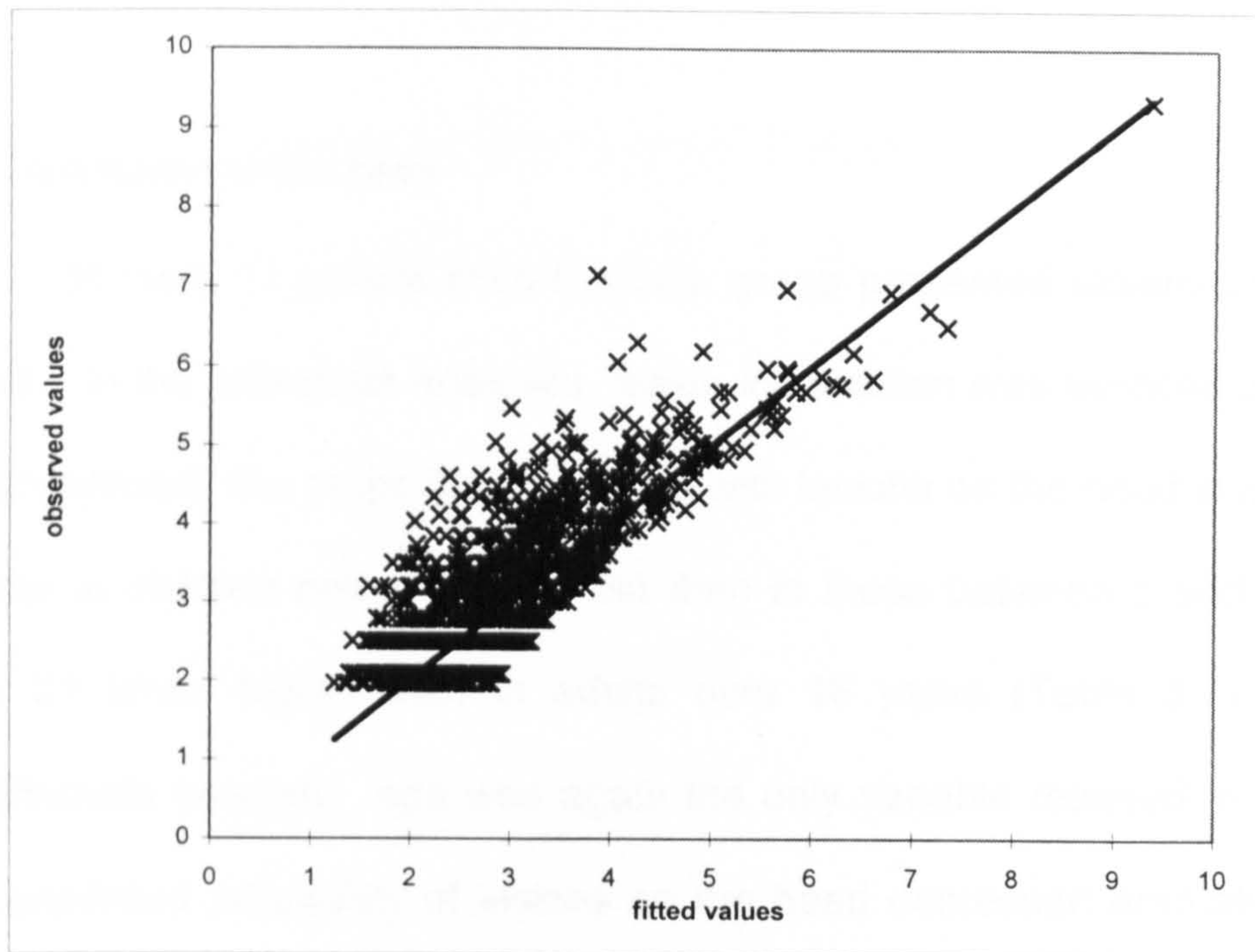
b) Number of scars

The overall geometric mean number of scars was 2.1, ranging from 1 to 30 scars ($n = 1187$). The univariate analyses demonstrated that the number of scars differed significantly with respect to gender, localisation on the body, "age now", age when infected and scar area (Table 3.6): (1) scarred females had more scars than scarred males; (2) people with scars on the trunk or legs (combined geometric mean 1.7 scars, range 1-20) had significantly more scars than people with scars on the head or arms

(combined geometric mean: 1.5 scars, range: 1-14) (t test = 3.65; D.F. 1,795; $P < 0.001$); (3) people over 15 years of age both when infected and “now” had less scars; and (4) people with larger scars ($> 3 \text{ cm}^2$) had more scars.

In the MAM generated by multivariate analysis scar number was associated with scar area, “age now” and gender. We can illustrate the change in the predicted scar number associated with changes in “age now” by concentrating on males with a scar area of 2 cm^2 (the population mean). A change in “age now” from 1 to 50 years is associated with a decrease in scar number from 2.4 to 1.6 scars. In contrast, a change in the maximum scar area from 2 to 10 cm^2 , on males with “age now” of 24 years (the population mean), is associated with a remarkable increase in scar number from 2 to 12 scars. For people with average age and average scar area, the model predicts 2.4 scars for females and 2 for males. In summary, scar number is predicted to increase with the area of scar but decreases with “age now”. Females are likely to have more scars than males. This model explains 10.5% of the variance in scar number (Figure 3.3)

Figure 3.3 The relationship between the observed and fitted number of scars according to the MAM



3.2.3.3 Risk factors associated with the localisation on the body of lesions or scars

Both univariate and multivariate analyses were carried out on persons with lesions (LCL group) or scars (scarred group) located only on one part of the body (i.e. head, trunk, arms or legs), having region, gender, age now (current age for scarred group) and age when infected (LCL and scarred) as explanatory variables. Localisation was treated as a binary response

variable in the multivariate analyses. 122 LCL patients and 1,187 scarred people comprised the two datasets for these analyses.

a) Localisation on the head

In total, 11 people from the LCL group presented lesions only on the head. In the univariate analyses, lesion localisation was associated with age when infected: the proportion of people with lesions on the head was 12 times higher in children below 6 years old than in those between 6 and 15 years, and 21 times higher than in adults over 16 years (Table 3.7). In the multivariate analysis, age was again the only variable retained in the MAM: the predicted probability of lesions on the head decreased dramatically from 0.16 at 1 year of age to 0.03 at the age of 10 years (Table 3.8 and Figure 3.4).

In the univariate analyses of the scarred group ($n = 115$), localisation was associated with geographic region, "age now" and age when infected: (1) there was a higher proportion of people with scars on the head amongst the scarred population in the south east part of the study area (regions 3, 4, 5 and 6; i.e. La Soledad, Miralindo, Valparaiso, San Pedro, Tagual and Yolandas) than in region 1 (i.e. Buenos Aires, Cucuchonal and Plan de Armas); (2) as for LCL patients, younger scarred people were more likely to

have scars on the head than were scarred adults, in relation both to “age now” and age when infected (Table 3.7).

In the multivariate analyses, geographic region and age when infected were retained in the MAM (Table 3.8): In region 1 and 2 (Figure 3.5) the expected probability of scars on the head decreased significantly with age from 0.26 at one year of age to 0.047 at 50 years old . In regions 3 to 6, the probability of scars on the head decreased from 0.12 to 0.02 in the same age range. However, this model explains only 5.5% of the variance in the probability of lesion localisation on the head.

b) Localisation on the arms

Amongst the LCL group, 55 people presented lesions on the arms. In the univariate analyses, region and gender were associated with localisation (Table 3.7): (1) a greater proportion of patients from region 3 (La Soledad) presented lesions on their arms than did patients from other regions; and (2) scarred males presented more lesions on the arms (52%) than scarred females (46%). However, in the multivariate analysis, gender and geographic region drop out from the maximal model and only age was retained in the MAM: the predicted probability of lesions on arms increased significantly with age, from 0.38 for 1 year old to 0.78 for 50 year olds (Table

3.8;Figure 3.6). This model explains only 8.8% of the variance in the probability of lesion localisation on the arms.

Both in the univariate and multivariate analyses of the scarred group, no risk factor was detected which predicted the probability of scars on the arms.

c) Localisation on the legs

Both in the univariate and multivariate analyses of LCL patients, no risk factor was detected which predicted the probability of lesions on the legs.

In the univariate analyses of the scarred population the probability of scar localisation on legs was associated with region, gender, “age now” and age when infected: (1) people in region 5 (San Pedro and Tagual) were more likely to have scars on the legs than people from other regions; (2) a greater proportion of scarred females had scars on the legs than did males (3) the proportion of scarred population with scars on the legs increased both with age when infected and with “age now”.

In the multivariate analysis “age now”, gender and geographic region were retained in the MAM: Scarred females had 1.8 times more scars on the legs than scarred males and this tendency increases with age. However,

there was significant differences between regions 1 and 5 with the other regions: The probability of scars located on the legs for females living in regions 1 and 5 increases from 0.49 at one year of age to 0.60 at 50 years, whilst for females in regions 2,3,4 and 6 the increase is from 0.37 to 0.48 for the same age range (Figure 3.7 and Figure 3.7). This model explains only 3.8% of the variance in the probability of lesion localisation on the legs.

d) Localisation on the trunk

In both univariate and multivariate analyses of the LCL group, no significant risk factors were detected for predicting the probability of lesions located on the trunk.

Amongst the scarred group, 56 people presented scars on the trunk. In the univariate analyses, only gender was associated with localisation: scarred males were more likely to have scars on the trunk (30%) than were scarred females (9%) (Table 3.7). However, in the multivariate analysis gender, "age now" and age when infected were retained in the MAM: For a 5 year old scarred child infected within the previous year, the probability of a scar located on the trunk is 0.10 whilst for a female in similar conditions the probability is only 0.048. These probabilities increases with age when infected but decreases with "age now" (Table 3.8). The MAM explains only 8 % of the variance in the probability of lesion localisation on the trunk

Table 3.7 Risk factors associated with the localisation on the body of lesions or scars (univariate analyses)

LESIONS		HEAD		ARMS		LEGS		TRUNK	
EXPLAN. VARIABLES	total	n	STATISTICS	n	STATISTICS	n	STATISTICS	n	STATISTICS
VILLAGE	1	4		16		15		1	
	2	2		13		6		1	
	3	2	$X^2 = 1.37$	17	$X^2 = 11.86$	4	$X^2 = 10.59$	1	$X^2 = 2.190$
	4	1	$P > 0.05$	1	$P < 0.05$	2	$P > 0.05$	0	$P > 0.05$
	5	1	D.F = 5	4	D.F = 5	11	D.F = 5	2	D.F = 5
	6	1		6		3		1	
GENDER	FEMALE	6	$X^2 = 0.03$	33	$X^2 = 0.24$	22	$X^2 = 0.09$	3	$X^2 = 0.01$
	MALE	5	$P = 0.86$	22	$P = 0.65$	19	$P = 0.75$	3	$P = 0.93$
AGE	<6	10	$X^2 = 11.19$	17	$X^2 = 2.03$	16	$X^2 = 0.25$	4	$X^2 = 0.117$
	6-15	1	$P < 0.01$	21	$P = 0.36$	18	$P > 0.05$	1	$P > 0.05$
	>15	0	D.F = 1	10	D.F = 1	7	D.F = 1	1	D.F = 1
SCARS									
VILLAGE	1	12		69		80		9	
	2	15		59		50		11	
	3	26	$X^2 = 17.06$	45	$X^2 = 5.53$	46	$X^2 = 12.21$	8	$X^2 = 6.27$
	4	25	$P = 0.004$	52	$P = 0.35$	44	$P = 0.031$	15	$P = 0.28$
	5	19	D.F = 5	44	D.F = 5	69	D.F = 5	10	D.F = 5
	6	18		34		34		3	
GENDER	FEMALE	52	$X^2 = 3.01$	147	$X^2 = 3.53$	198	$X^2 = 10.9$	18	$X^2 = 9.72$
	MALE	63	$P = 0.082$	156	$P = 0.06$	125	$P = 0.0009$	38	$P = 0.0018$
AGE NOW	<6	15	$X^2 = 14.16$	20	$X^2 = 1.49$	14	$X^2 = 6.69$	5	$X^2 = 2.4$
	6-15	47	$P = 0.0008$	94	$P = 0.47$	103	$P = 0.035$	23	$P = 0.3$
	>15	53	D.F = 1	189	D.F = 1	206	D.F = 1	28	D.F = 1
AGE INF	<6	53	$X^2 = 23.44$	81	$X^2 = 0.65$	71	$X^2 = 9.04$	16	$X^2 = 0.3$
	6-15	37	$P = 0.0000081$	115	$P = 0.72$	133	$P = 0.01$	23	$P = 0.86$
	>15	25	D.F = 1	107	D.F = 1	119	D.F = 1	17	D.F = 1

significant variables

Table 3.8 Minimal Adequate Model for risk factors associated with area, number and localisation of scars and lesions

OUTCOME	MODEL	PARAMETER	estimate	s.e	statistics
AREA	LCL	intercept (flat border)	0.1787	0.09285	F = 9.7 D.F. ,2,165 P < 0.01 $r^2 = 10.5$
		raised border	0.6586	0.1729	
		verrucosa	1.42	0.4568	
	SCARS	intercept (mult. les.)	1.064	0.02192	$r^2 = 18$ F=36.27;D.F.1,1182;P<0.001 F=22.68;D.F.1,1182;P<0.001 F=14.68;D.F.1,1182;P<0.001 F=130.3;D.F.2,1183;P<0.001 F=130.3;D.F.2,1183;P<0.001
		number of scars	0.0165	0.002739	
		age now	0.003208	0.000673	
		age when infected	-0.00351	0.0009	
		site head+arms+trunk	-0.1451	0.01978	
		site legs	0.06908	0.02112	
NUMBER	LCL	-	-	-	-
	SCARS	intercept	0.6226	0.09943	$r^2 = 10.5$ $\chi^2=26.56$;D.F1;P<0.001 $\chi^2=8.8$;D.F1;P<0.01 $\chi^2=71.5$;D.F1;P<0.001
		age now	-0.00871	0.001732	
		gender (male)	-0.1673	0.05652	
		area	0.2253	0.02564	
HEAD LOCALIS.	LCL	intercept	-0.9871	0.4627	$\chi^2 = 11.19$ 1 D.F. $r^2 = 15.6$ P < 0.01
		age	-0.2132	0.09495	
	SCARS	intercept (reg.,3,4,5,6)	-1.875	0.226	$r^2 = 5.5$ $\chi^2=17.87$;D.F1;P<0.001 $\chi^2=15.52$;D.F1;P<0.001
		age when infected	-0.04012	0.01034	
		region 1+2	0.8825	0.2356	
ARMS LOCALIS.	LCL	intercept	-0.5061	0.2667	$r^2 = 8.8$ $\chi^2=5.31$;D.F1;P<0.05
		age now	0.03641	0.01662	
	SCARS	-	-	-	-
LEGS LOCALIS.	LCL	-	-	-	-
	SCARS	intercept (villages 1+5)	-0.03761	0.1745	$r^2 = 3.8$ $\chi^2=18.73$;D.F1;P<0.001 $\chi^2=4.895$;D.F1;P<0.05 $\chi^2=10.35$;D.F1;P<0.01
		gender (male)	-0.6377	0.1484	
		age now	0.00941	0.00415	
		villages 2+3+4+6	-0.4832	0.1503	
TRUNK LOCALIS.	LCL	-	-	-	-
	SCARS	intercept	-2.756	0.311	$r^2 = 8$ $\chi^2=8.4$;D.F1;P<0.01 $\chi^2=6.359$;D.F1;P<0.05 $\chi^2=8.712$;D.F1;P<0.01
		age now	-0.04458	0.01727	
		age when infected	0.04864	0.02005	
		gender (male)	0.8492	0.297	

Figure 3.4 Probability of lesions on the head by age

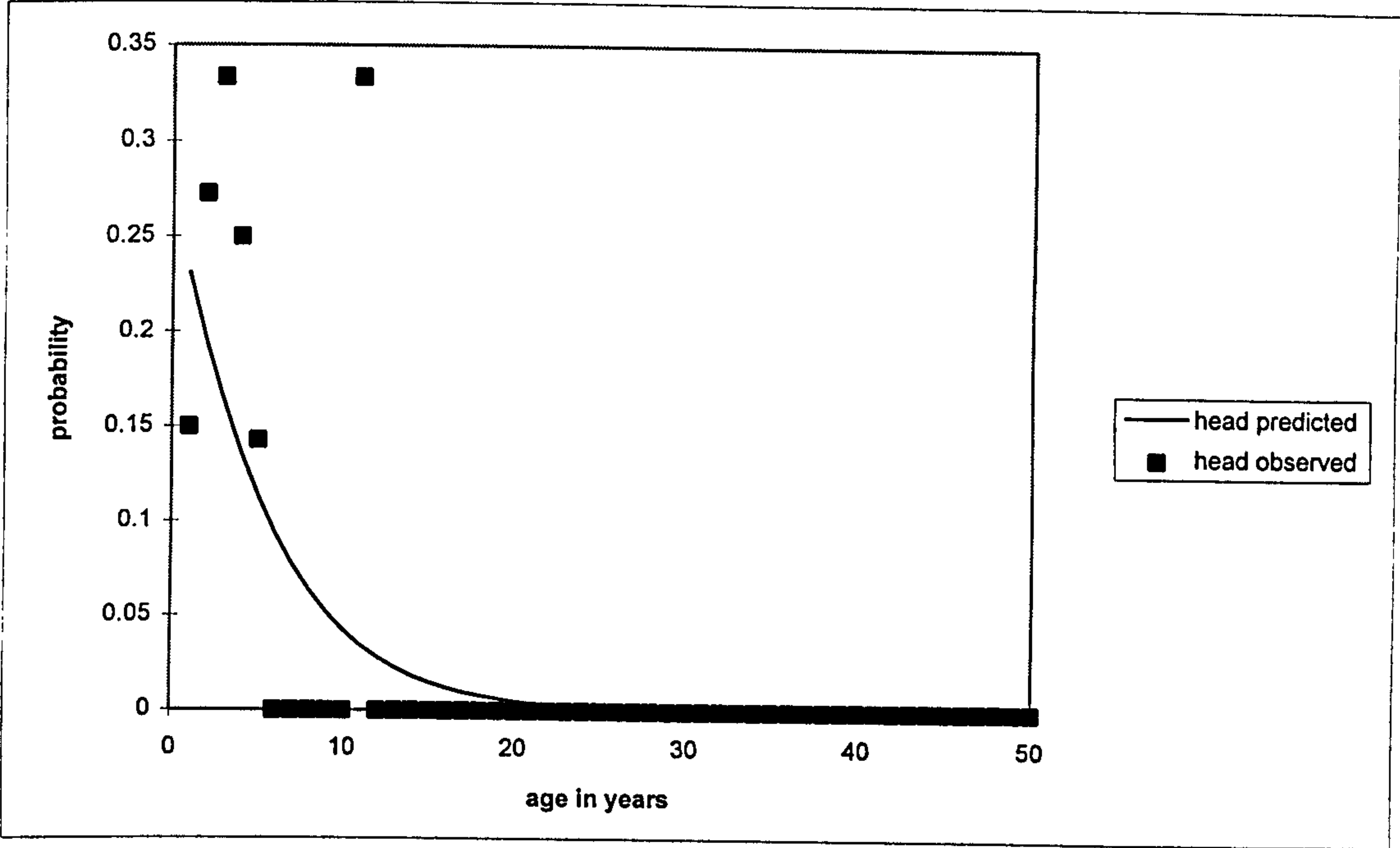


Figure 3.5 Probability of scars on the head by age when infected (in regions 1 and 2)

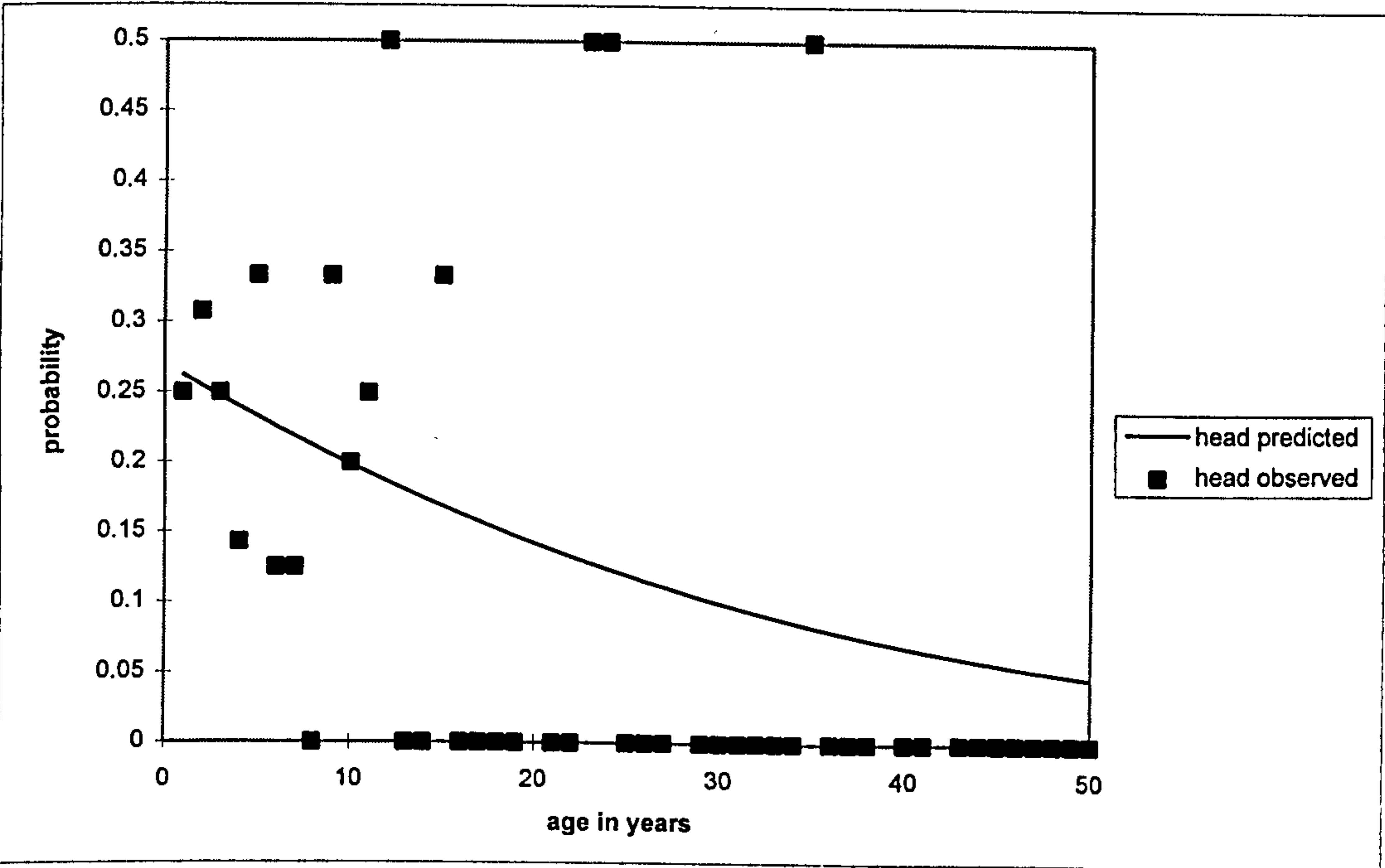


Figure 3.6 Probability of lesions on the arms by age

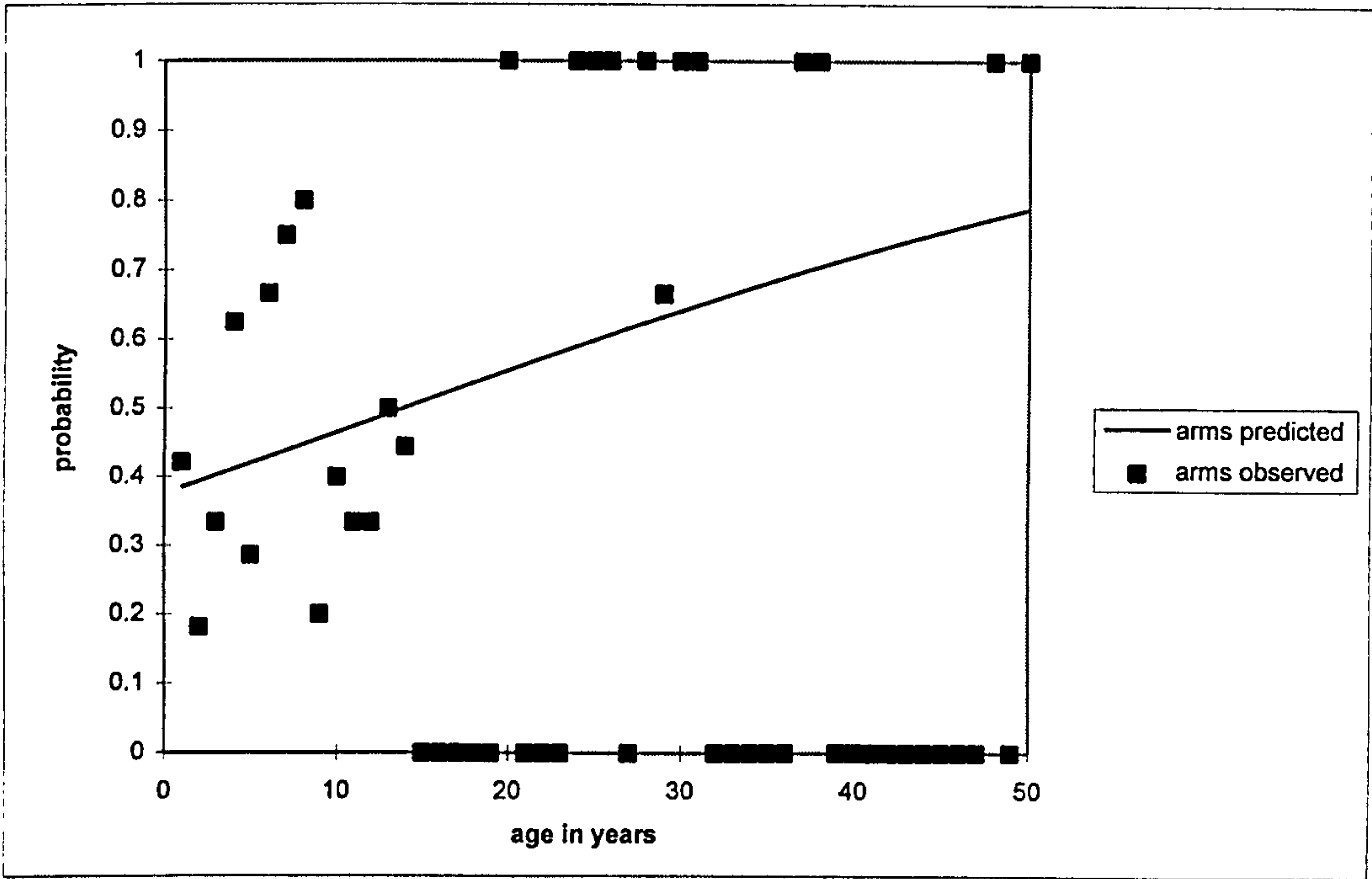


Figure 3.7 Probability of scars on the legs by gender (in regions 1 and 5)

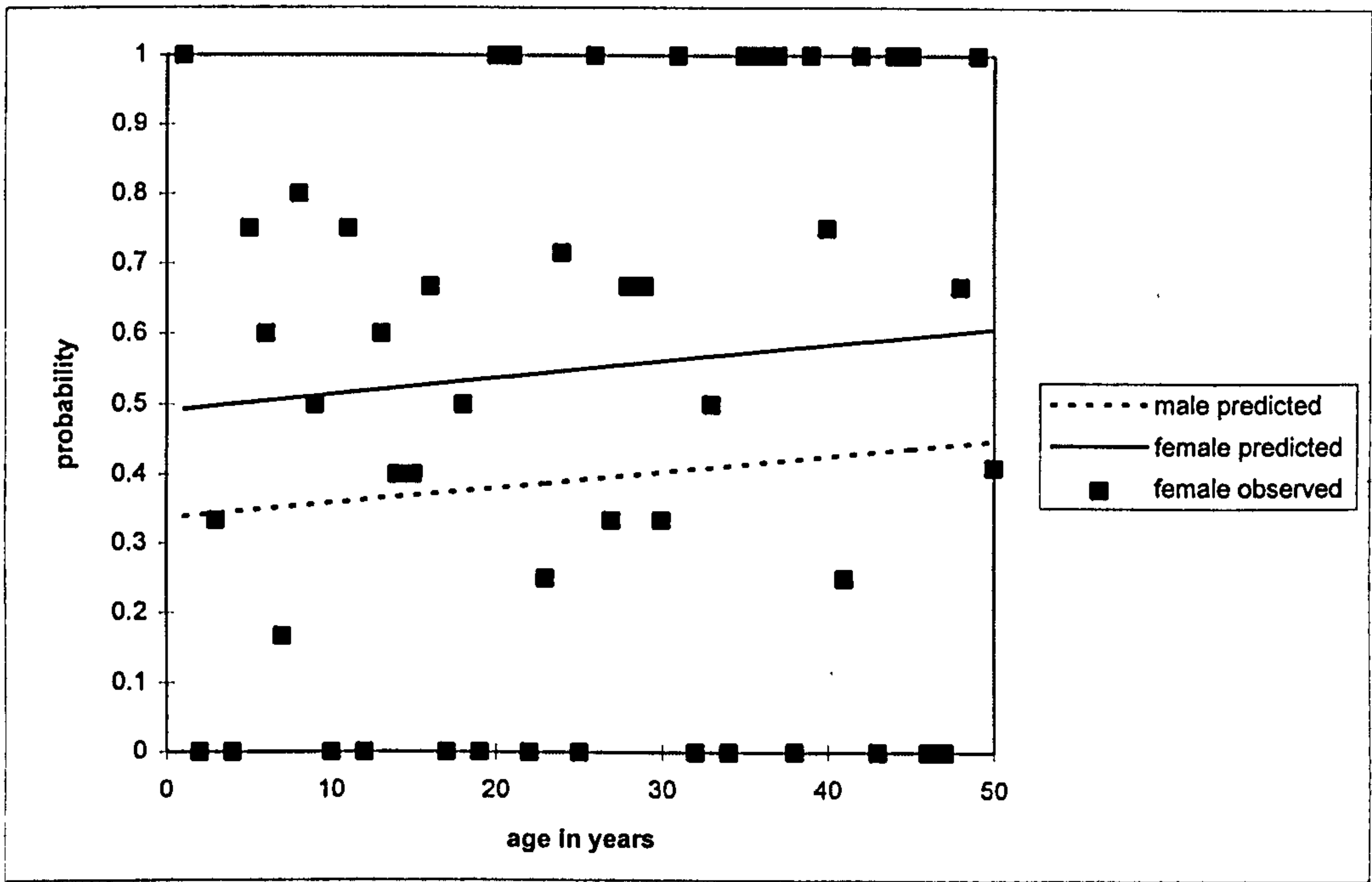
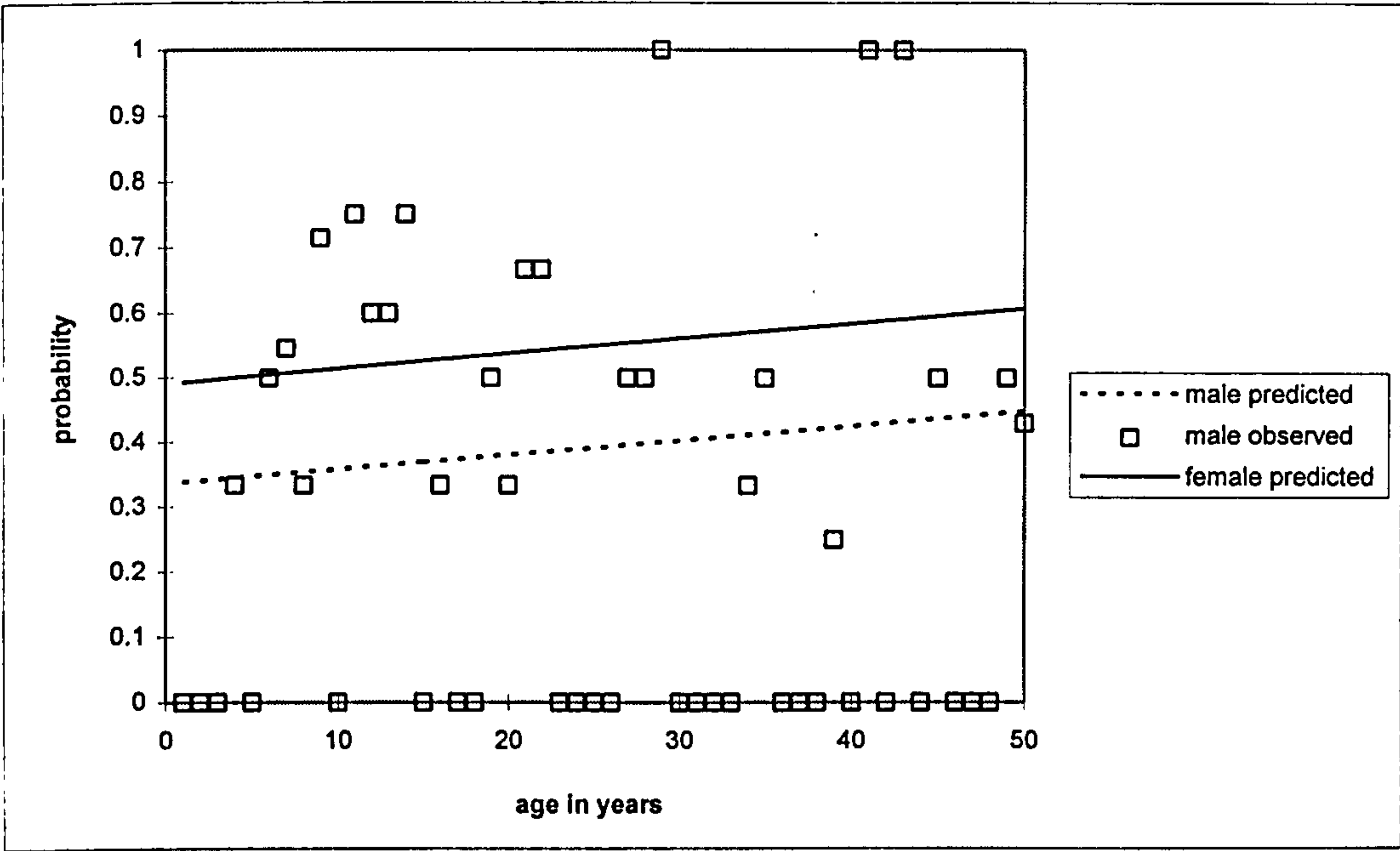


Figure 3.8 Probability of scars on the legs by gender (in regions 1 and 5)



3.2.4 Comparison of patients in relation to the results of parasitological diagnosis and spontaneous healing rate

Clinical histories were recorded and parasitological and molecular diagnostic tests carried out on 168 patients in the study site. These were divided into three groups according to the results of their diagnostic tests (P+ positive, or P- negative), and whether or not they healed within 3 months from enrolment (H+, or H-). The first group comprised 44 P-H+ clinically suspected patients who had consistently negative results for all diagnostic tests and who spontaneously healed within 3 months of their first examination. The second group comprised 94 P+H- patients with a positive parasitological or molecular diagnosis, and who required treatment with

Glucantime®. The third group comprised 30 P-H- clinically suspected patients with persistent negative results, but who also had persistent lesions, i.e. they failed to heal spontaneously within 3 months from enrolment.

Risk factors that determined the results of the diagnostic tests were sought by comparing the parasitologically positive patients (P+) with P-, both in univariate and multivariate analyses. The following factors were tested: (1) clinical appearance of the lesions (i.e. type, localisation, description, and presence of secondary infection or lymphadenitis), (2) patients' provenance (village), (3) personal factors (i.e. age, gender) and (4) presence of previous infections. The same risk factors were then tested (both by univariate and multivariate analyses) for their ability to predict whether or not a patient healed spontaneously.

3.2.4.1 Risk factors for a positive parasitological diagnosis

The overall rate of positive diagnosis for patients was 55.9% (94/168). In the univariate analyses P+ and P- differed significantly with respect to the type and localisation of the lesion, the presence of previous scars and patient age (Table 3.9). A positive parasitological diagnosis was significantly more likely for patients with ulcers (64%) or multiple types of lesions (69%), and for patients with lesions located on the trunk (83%) or on more than one part of

the body (72%). A positive diagnosis was also significantly more likely in younger patients, and in patients with no previous scar.

However, when those patients who healed spontaneously (H+), are excluded from the analyses, i.e. univariate comparisons are made between P+H- and P-H-, the only significant risk factor for a positive diagnosis is lesion type: a patient with multiple lesions was significantly more likely to have a positive diagnosis ($\chi^2 = 15.21$; D.F.. 4; $P = 0.004$). In contrast, comparisons between P-H+ and P+H- identified several risk factors: (1) the geometric mean age was significantly higher in P-H+ (11.6 years) than in P+H- (6 years) (t test: 3.17, $P = 0.0018$); (2) lesions on multiple body sites were significantly more common amongst P+H- (33/46, 72%) than amongst P-H+ (4/46, 9%) ($\chi^2 = 11.93$; D.F.. 4; $P = 0.017$); and (3) P-H+ were significantly more likely than P+H- to have previous scars (50% vs 33%) ($\chi^2 = 8.45$; D.F.. 1; $P = 0.003$). A multivariate analysis of potential risk factors for a positive diagnosis resulted in a MAM containing the following explanatory variables: (1) lesion type ($\chi^2 = 5.87$; D.F.. 1; $P < 0.05$) and (2) presence of scars ($\chi^2 = 9.88$; D.F.. 1; $P < 0.01$). The presence of previous scars decreases the odds of a positive diagnosis by 25% (95% C.I. 11 - 45%), whilst the odds of a positive diagnosis for a patients with ulcer, papule, bubonic or multiple lesion is 66% (95% C.I. 57 - 74%) and for a patient with nodule is 22% (95% C.I. 11 - 39%).(Table 3.10)

Table 3.9 Risk factors associated with spontaneous healing and parasitological positive diagnostic tests

QUALITATIVE VARIABLES		TOTAL	SPONTANEOUS HEALING			PARASITOLOGICAL POSITIVE		
			n (H+)	% H+	statist.	n (P+)	% P+	statist.
REGION	1	43	10	23.3	$X^2 = 4.29$ D.F. = 5 P = 0.50	26	60.5	$X^2 = 2.12$ D.F. = 5 P = 0.83
	2	37	6	16.2		21	56.8	
	3	36	11	31		21	58	
	4	8	2	25		4	50	
	5	29	9	31		16	55	
	6	15	6	40		6	40	
GENDER	MALE	76	21	28	$X^2 = 0.69$	39	51	$X^2 = 1.2$
	FEMALE	92	23	25	DF.1,P=0.7	55	60	DF.1,P=0.2
TYPE OF THE LESION	PAPULE	28	8	29	$X^2 = 1.58$ D.F. = 4 P = 0.81	16	57	$X^2 = 12.16$ D.F. = 4 P = 0.016
	NODULE	32	10	31		10	31	
	ULCER	64	14	22		41	64	
	BUBONIC	15	5	33		7	47	
	MULTIP.TYPE	29	7	24		20	69	
LOCALIZATION	HEAD	11	3	27.2	$X^2 = 11.19$ D.F. = 4 P = 0.024	5	45	$X^2 = 9.36$ D.F. = 4 P = 0.05
	UPPER EXTR.	57	19	33.3		27	47	
	LOWER EXTR.	48	17	35.4		24	50	
	TRUNK	6	1	16.6		5	83	
	MULTIPLE LES.	46	4	9		33	72	
PREVIOUS SCARS	NO	144	32	22	$X^2 = 8.16$	86	60	$X^2 = 5.78$
	YES	24	12	50	D.F = 1	8	33	D.F. = 1
					P = 0.004			P = 0.016
DESCRIPTION OF THE ULCER	FLAT	116	34	29	$X^2 = 1.89$	61	53	$X^2 = 2.96$
	RAISE	47	9	19	D.F. = 2	31	66	D.F. = 2
	VERRUCOSA	5	1	20	P = 0.38	2	40	P = 0.22
OTHERS	NONE	111	31	28	$X^2 = 1$	59	53	$X^2 = 1.62$
	SECOND. INF.	27	5	19	D.F. = 2	18	67	D.F. = 2
	LINFADENITIS	30	8	27	P = 0.6	17	57	P = 0.44

QUANTITATIVE VARIABLES geo.mean(\pm C.I.)	SPONTANEOUS HEALING				PARASITOLOGICAL POSITIVE			
	n = 44	n = 124	t test	P	n = 94	n = 74	t test	P
	H+	H-			P+	P-		
AGE (95% C.I.)	11.6 9.9-15.1	6 4.8-7.5	3.22	0.0015	6 4.5-7.7	9 7.0-12.6	2.22	0.027
AREA (95% C.I.)	1.5 1.0-3.0	2 1.6-2.5	1.66	0.097	2.1 1.7-2.5	1.6 1.1-2.2	1.46	0.14
EVOLUTION (95% C.I.)	3.8 2.7-5.2	3 2.5-3.7	1.16	0.24	2.5 2.0-3.0	3.4 2.7-4.4	1.85	0.06
NUMBER (95% C.I.)	1.7 1.3-2.0	2 1.8-2.2	1.56	0.11	2 1.8-2.2	1.8 1.5-2.1	0.92	0.35

Table 3.10 Minimal adequate models for predicting spontaneous healing and a positive parasitological diagnosis

MODEL	PARAMETER	estimate	s.e
PARASITOLOGICAL POSITIVE PATIENTS	Intercept (ulcer, nodule, papule, and bubonic)	0.6445	0.1921
	previous scars	-1.122	0.4755
	nodule	-1.293	0.4254
SPONTANEOUS HEALING	Intercept (head, lower, upper, trunk)	-0.9365	0.2143
	multiple places	-1.684	0.5685
	previous scars	1.322	0.4806

3.2.4.2 Risk factors for a chronic lesion

Forty four patients (26.1%) healed spontaneously in a period ranging between one and three months after the patient diagnostic procedures began. In the univariate analyses, H+ and H- patients differed significantly with respect to localisation of the lesion, the presence of previous scars and patient age (Table 3.9). Spontaneous healing was significantly more likely to occur for patients whose lesions were located on only one part of the body (32%) (upper or lower extremities, head or trunk) than for patients with lesions on multiple body sites (9%) (odds ratio, 5.12; 95% C.I. 1.67 - 20.87; $\chi^2 =$

9.97; $P < 0.001$). Spontaneous healing occurred significantly more frequently in older patients, and in patients with previous scars. When univariate comparisons are made between P-H+ and P-H- the only risk factor for chronic lesions was age: the geometric mean age was significantly higher in P-H+ (11.6 years) than in P-H- (7 years) (95% C.I. 3.8 - 9.6 years) (t test: 2.52, $P = 0.013$). A multivariate analysis of potential risk factors for a chronic lesion resulted in a MAM containing the following explanatory variables: (1) localisation of the lesion ($\chi^2 = 11.80$; D.F.. 1; $P < 0.001$) and (2) presence of scars ($\chi^2 = 7.53$; D.F.. 1; $P < 0.01$). The presence of previous scars increases the odds of a spontaneous healing by 79% (95% C.I. 59 - 90%), whilst the odds of spontaneous healing for a patient with lesions located on more than one place is 16% (95% C.I. 6 - 36%) and for a patient with a unique lesion is 28% (95% C.I. 20 - 37%). (Table 3.10)

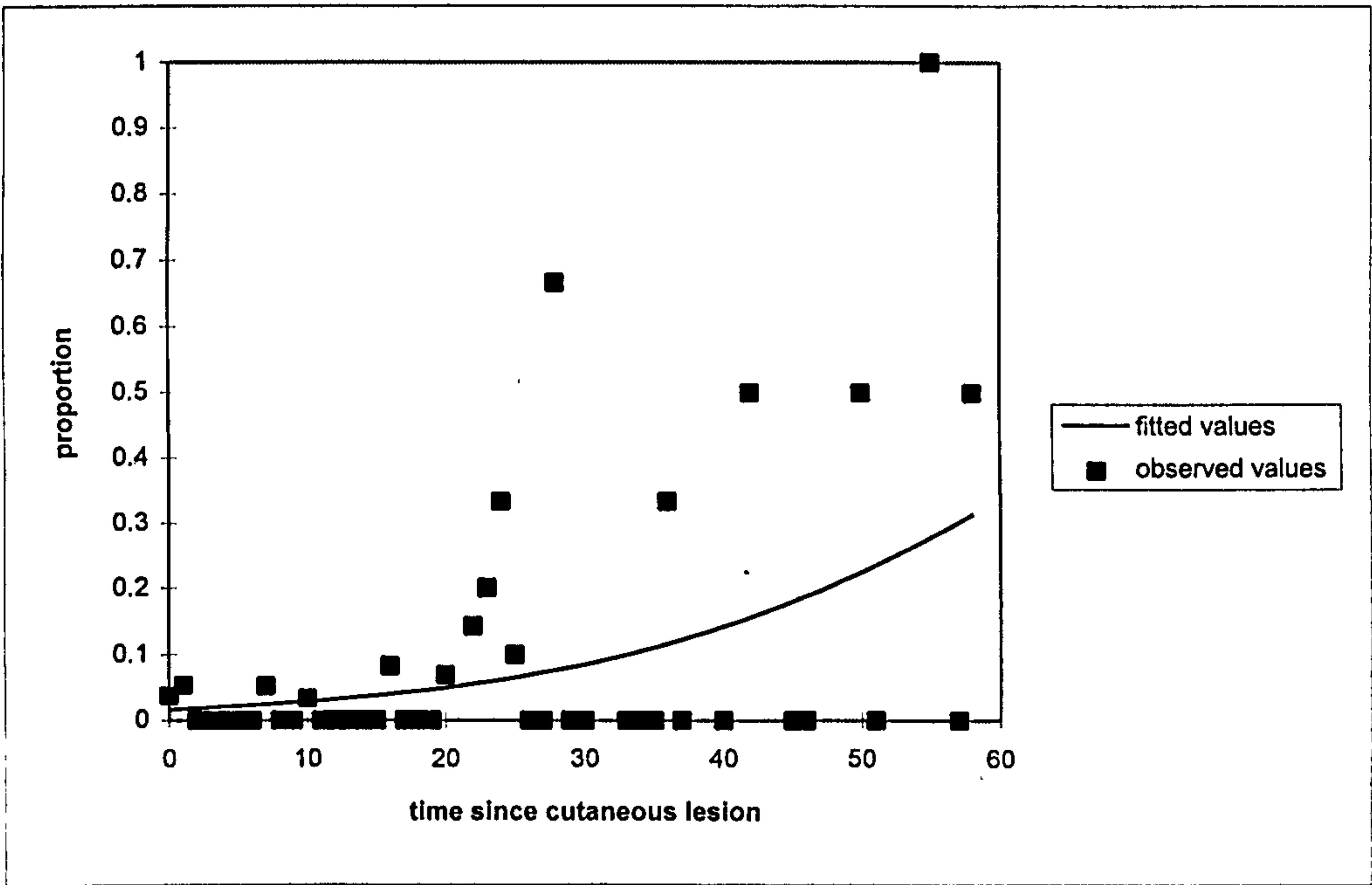
Figure 3.2 The role of spontaneous healing

3.2.5 Mucosal leishmaniasis (MCL)

Clinical inspections for mucocutaneous leishmaniasis were carried out in the study population in 1,100/1,380 people, from which 92 presented lesions in the nasal septum: 27 patients presented perforations and the other 65 patients presented ulcers. PCR was carried out on all 92 samples taken from patients, but the method chosen for DNA preservation (NET 10 SDS) did not prevent the denaturation of the DNA in the majority of the samples. Only

two samples from the group highly suspected by clinical grounds (perforated nasal septum) were positive by PCR. In total, 2.9% (27/938) of all LCL patients (past or present) developed perforated septa; whilst an additional 7.3% (69/938) developed nasal ulcers. Hence there was evidence that a total of 10.2% of all LCL patients had developed a low severity mucosal lesion. A significant association between the time since the cutaneous lesion and the appearance of MCL was detected by a logistic regression of the results for the group of patients with perforated septum. The curve depicts a smooth increase in MCL with time ($\chi^2 = 28.6$; 1 D.F.; $P < 0.001$) (Figure 3.9). However, no significant association was detected between the time since the cutaneous lesion and the presence of a nasal ulcer (indicating that ulcers might self-heal)

Figure 3.9 The rate of suspected metastasis



3.3 DISCUSSION

3.3.1 DIAGNOSTIC METHODS

In the following sections, the relative sensitivity of the different diagnostic tools used in the Opon study, are compared with the results of previously reported comparative studies. However, it is important to stress that comparison of the sensitivity of different diagnostic tests may generate inconsistent results in different field studies, in part because of parasite heterogeneity, and also because of variation in the average evolution time of the lesions prior to diagnosis. For example, in Tumaco (Weigle et al, 1987), the sensitivity of all diagnostic methods used (including dermal scraping, histopathology, and cultures) decreased approximately by 50% in lesions with an evolution time longer than 6 months.

Parasitological methods are the routine tools commonly employed in the diagnosis of cutaneous leishmaniasis in Central and South America. They included the microscopic examination of slides, the isolation of parasites in artificial media or in hamsters, and histopathology. In previously reported studies the measurement of sensitivity of these methods has taken clinical criteria as the "gold standard". For example, the sensitivity of seven parasitological methods in Colombia were evaluated on 165 patients, clinically suspected on the basis of a skin lesion of two or more weeks of

evolution and no history of trauma (Weigle et al, 1987). However, the sensitivity and specificity of the more novel molecular biology techniques (e.g. PCR) have generally been evaluated using parasitological diagnosis as the "gold standard" (de Bruijn et al, 1993).

3.3.1.1 Microscope examination (ME)

In studies involving the passive search of patients, more severe and chronic lesions are expected than in studies where patients are located by active search (as in follow-up studies) (Saravia & Weigle, 1996), and therefore the expected results of ME are low. The negative relationship between ME and the evolution time of the lesions was demonstrated by Herwaldt (1992) in Guatemala, where the amastigote concentration in smears from older lesions [median = 1 amastigote/100 oil immersion fields] was significantly lower than that in recent lesions [median = 17 amastigotes/100 oil immersion fields]. However, in the only two follow-up studies with active search that have now been carried out in Colombia, the ME sensitivity was considerably lower than the ME sensitivity in two Colombian studies involving the passive search of patients: i.e. in Opon and Tumaco (active search) ME sensitivity was 33% and 22% (Weigle et al, 1987), respectively; whilst in Antioquia and Norte de Santander (passive search) ME was 60% (Velez et al, 1987) and 55% (Corredor et al, 1987) respectively. Although there is no

previously reported difference in the ME sensitivity of samples taken from patients infected with *L. panamensis* or *L. braziliensis*, interpretation of the results of these Colombian studies should be made with caution: *L. panamensis* was the main parasite found in Opon and Tumaco, whilst *L. braziliensis* was found in the other two foci.

Two factors which may effect the measurement of ME sensitivity are the type of instrument used, and the site in the ulcer where the sample was taken. In the Opon focus, samples were taken from the edge of the ulcer after an incision in the skin with a scalpel, because this method reduces the probability of secondary bacterial infection in the patient. An investigation of ME sensitivity was carried out in Guatemala (Navin et al, 1990), comparing the use of capillary tubes, scalpels or dental broaches to collect samples, and the border or centre of the ulcer as the site for taking samples. No significant differences in sensitivity were observed, and so it is reasonable to assume that the relatively low sensitivity in the Opon study was not due to the use of scalpel, nor to the selection of the site in the body where the sample was taken.

There are a considerable number of unreported works describing high sensitivity for ME in patients attending private and public hospitals (passive search) in Colombia; and these reports have significantly influenced the policy making decisions of the Colombian Ministry of Health. At present, ME is the

recommended “first line” technique in Colombia, because it is easy, cheap and sensitive. If the ME result is negative, histopathology is recommended. However, considering both the Opon and Tumaco results, ME cannot be recommended for diagnosing *L. panamensis*, and histopathology demonstrated even lower sensitivity than ME in Tumaco. Thus, this thesis should stimulate the revision of Colombian health policy with respect to leishmaniasis diagnosis.

3.3.1.2 Culture of parasites

L. panamensis is considered “the most easily cultivated member of the *L. braziliensis* complex” (Walton et al, 1977). The culture of aspirates taken from the border of the lesion is one of the diagnostic methods with the highest reported sensitivity. Hendricks and Wright (1979) reported 67% recovery of *L. panamensis* using *in vitro* cultivation of saline aspirates in Schneider’s *Drosophila* medium; and in Tumaco the parasite isolation rate was 58%. In Opon, using the same procedures as in Tumaco (see Materials and Methods), the sensitivity was consistently lower (33%), even though contamination of samples was relatively infrequent and the parasites in both foci were *L. panamensis*. The most probable explanation for the low rate of parasite isolation in Opon is the exposure of cultures to changes in temperature during field visits (even though tubes were maintained in the best

conditions available), because samples were taken at people's homes, rather than at a central health post. So parasite isolation should only be recommended in combination with other first line tools, when patients are willing to visit the health posts. If diagnostic attempts can only be made during the house-to-house visit by a community "active search team", the ME and / or PCR (see below), but not isolation, should be carried out.

3.3.1.3 Polymerase Chain Reaction (PCR)

In the Opon focus, PCR was tested as an alternative method for the detection of parasites in human and sandfly samples (see Chapter 5). In previous studies, PCR has demonstrated low specificity, i.e. a relatively high percentage of patients with non leishmanial aetiologies were PCR positive (e.g. 50% in Colombia and 69% in Peru: deBrujin et al, 1993, and Lopez et al, 1993, respectively), But PCR has been shown to have a reasonably high sensitivity (80% in Colombia and Peru), when compared with the results of standard parasitological methods. In Opon, PCR specificity was not measured because this technique was only used in samples from clinical suspected patients, but the sensitivity when compared with the other parasitological methods was high (87%, Table 3.2).

Apart from manipulation errors when samples are taken from patients, the processing of samples by long procedures (i.e. DNA extraction) could be

one of the major causes of cross-contamination of DNA between positive and negative samples, so reducing the specificity of PCR as a diagnostic tool. In the Opon focus, all samples were taken from patients with disposable instruments, stored in dry conditions and processed by water lysis, which reduces DNA contamination. However, in future studies, PCR specificity should be measured in a large number of negative skin samples from people living in an area free of leishmaniasis (Berman, 1997) and with no history of visits to endemic areas. For example, skin samples could be tested from the fresh corpses of recently deceased people in San Andres Island, Colombia, where no leishmaniasis cases have ever been reported.

Assuming that PCR is a favourable option in the future, the Opon results can be extrapolated to indicate the likely success of different diagnostic strategies involving the active search of patients. The best estimate of sensitivity was 61.9%, when all three methods were used, followed by either combination : (1) PCR - Giemsa or (2) Giemsa - culture (both 57%) (Table 3.3). Following the Opon example, it is recommended that future community based networks for leishmaniasis diagnosis should be composed of one person per village (a local trained farmer), the local health post, and a central laboratory. Samples taken in each village should be sent to the local health post for ME, and negatives samples should be sent to the central laboratory for PCR. This routine should not to be applied to cultures, as the manipulation, storage and transport will undoubtedly decrease the

probability of isolation to even lower levels than those detected in Opon. Thus, the most effective combination of diagnostic tools for parasite detection should be Giemsa - PCR. As seen in Materials and Methods, the simplicity of PCR procedures should make it an affordable health policy for the detection of leishmaniasis cases by the active search of patients. Microscopically examined negative samples from villages can be sent to the central lab by normal post with no special equipment required. But, as for the current procedures as histopathology, there will be an urgent requirement for the result to be reported back to the local hospital within a minimum number of days.

3.3.2 PARASITE CLASSIFICATION

Isoenzyme electrophoresis was used for the classification of human isolates rather than for the detection of genetic polymorphism in the parasite population in Opon. All the 25 parasites isolated and characterised during this project were typed as *L. panamensis*. However, previous isolates made in the focus have been characterised as other species: *L. braziliensis* and *L. colombiensis*, which was isolated for the first time from an infected sandfly (*Lu. hartmanni*) in the municipality of El Carmen, only 80 Km from the Opon area (Kreutzer et al, 1991). Given the low isolation rate achieved, I cannot discount the likelihood that *L. braziliensis* and/or *L. colombiensis* also circulate

in Opon. In addition to enzymes commonly used for the identification of New World parasites, (e.g. GPI, MPI, 6PGDH), peptidase D (PEPD) was used for typing. Variation in the electrophoresis patterns of this enzyme was detected for 2 isolates. Polymorphism in this enzyme has rarely been detected amongst previously studied isolates (Kreutzer, 1987, 1996). The two isolates for which this enzyme varied came from patients in Plan de Armas, possibly suggesting some geographic heterogeneity amongst parasite populations in Opon. No variations were observed in the other enzymes, and there was no evidence of any hybrids between *L. panamensis* and other species, as those described previously between this parasite and *L. braziliensis* (Cupolillo, 1997). However, polymorphism of Opon parasites may be associated with distinct clinical manifestations, which in the future should be tested with more isolations.

3.3.3 CLINICAL FEATURES

In Opon, the mean size of the active lesions was slightly smaller than the mean size of previous scars. This is an indication of the potential benefits that would arise from the speedy diagnosis and treatment of suspected cases (as took place during this project). A reduction in the scar size would be especially important for people whose lesions are located on the face. The maximum scar size apparently decreased with “age when

infected” suggesting that adults are less susceptible than children and are therefore less prone to severe symptoms. Acquired immunity with age could also explain the observations that (1) parasitological diagnosis was more likely to be positive in children less than 10 years old, and (2) parasitological diagnosis was more likely to be negative in people with scars from a previous clinical infection. Hence, in general, children have a great risk of chronic lesions, which required an extensive course of treatment. In contrast, “age now” was positively correlated with scar size, which probably reflects the fact that as “age now” increases so does the time since infection, and scar size will increase with time as children grow (Davies et al, 1997a).

In Opon, the scar number tended to increase with scar area, which is contrary to the Peruvian results, where it was suggested that the immunological response in coincident lesions may act synergistically in reducing mean lesion size (Davies, 1997a). Probably the malnutrition in the Opon children is more acute than in Peru, and this factor could weaken their immune response (Dye & Williams, 1993). Parasitological diagnosis was more likely to be positive in patients with multiple types of lesions located in more than one part of the body.

Peoples' behaviour and the place of transmission both change with age. Intradomiciliary transmission is the main risk for children below 10 years, who typically receive infected bites on the face when asleep; but adults

are more likely to be bitten during some outdoor occupation. This is why the probability of lesions on the head decreased dramatically with age. In adults, the relative frequency of lesions located on the arms, legs and trunk differed according to gender. A relatively high frequency of lesions were located on the trunk of adult males, presumably associated with their working behaviour: e.g. harvesting cacao fruits without wearing a shirt. In contrast, adult females had a relatively high frequency of lesions on their legs presumably related to their age-related change in their type of clothing: older females tend to wear skirts.

Mucocutaneous leishmaniasis was confirmed parasitologically in only two patients, possibly due to the difficulties in the diagnostic procedures. However, there was many patients (27/92, 29% of MCL patients) with perforations of the nasal septum; and the fact that this condition was only detected in patients with scars suggest that this symptom was a direct result of *Leishmania* infection. Nevertheless, the clinical picture of MCL in Opon was never as severe as in other leishmaniasis foci where *L. braziliensis* is circulating (e.g. in Bolivia or Peru), where MCL can involve the total destruction of the nasal septum ("tapir nose"), and the involvement of other organs including larynx and vocal cords, and is in some cases fatal (Desjeux et al, 1987).

4. EPIDEMIOLOGY

4.1 INTRODUCTION

Spatial, seasonal and temporal changes in the transmission patterns of leishmaniasis caused by *L. panamensis* have been evaluated previously in only in one follow up study carried out in Colombia in the Pacific coastal lowlands of Tumaco municipality (Figure 1.1) (Weigle et al, 1993). The ecology in Tumaco is not representative of the ecology of the inter-Andean valleys, where leishmaniasis caused by the same parasite has been increasing in recent years as a result of the establishment of new settlements, given the fertile conditions of the soil and the increase of the human population size. However, the epidemiology of leishmaniasis in Opon should be representative of these new types of foci , which are becoming increasingly common in Colombia and other Andean countries.

The increase in the incidence of leishmaniasis cases in Colombia stimulated the formation, in 1994, of the National Leishmaniasis Control Program (NLCP) by the Colombian Ministry of Health, with general objectives: (1) to detect cutaneous leishmaniasis cases soon after the first symptoms in order to offer opportune diagnosis and treatment, (2) to establish a network

of cutaneous leishmaniasis diagnostic centres at the first level of patient attendance, and (3) to stop the increasing morbidity and mortality of visceral leishmaniasis.

The NLCP recommends that epidemiological studies of cutaneous leishmaniasis should be carried out in each Colombian Department, and that these studies should address the identification of personal and household risk factors of transmission including: age, gender, occupation, localisation of the house with respect to the surrounding vegetation and type of construction. The NLCP further suggest that these objectives should be met by the application of MST and clinical inspection of all suspected active cases, as well as a random sample (ca. 10%) of the population within a defined focus. Finally, the NPLC recommend that epidemiological studies should be carried out to identify risk factors, which may be amenable to prevention and control strategies in the studied foci.

The design, methodology and analysis of the epidemiological study carried out for this thesis, as detailed in this Chapter, were chosen in order to address the objectives of NPLC. This thesis is the second prospective study of leishmaniasis to be carried out in Colombia, and it provides valuable information on the temporal and spatial patterns of leishmaniasis transmission in Colombia. It is hoped that the thesis will be a resource for the NPLC in providing advice on data collection and statistical analysis. Thus, the

specific objectives of the epidemiological study of leishmaniasis in Opon were:

1. To measure the transmission rate in the focus, and determine whether the risk of human infection is related to age and / or gender.
2. To determine the risk factors for infection associated with household location, according to the surrounding vegetation, with special attention paid to the effect of deforestation.
3. To determine the seasonal patterns of transmission.
4. To describe the characteristic course of clinical infection in the focus with respect to MST conversion and recovery, the frequency of recurrent clinical leishmaniasis or mucosal involvement, and the development of acquired immunity.

4.2 RESULTS

4.2.1 Population structure

In 1995 the inhabitants of 12 villages within the Opon focus comprised 2,704 people with an age structure characteristic of developing countries (Figure 4.1 and Table 4.1). Amongst the inhabitants of the 12 villages, participation in the study ranged from 36% in the village of Miralindo to 70% in the village of Buenos Aires (Table 4.2). Overall, the MST was applied to 51% of the Opon population; hence, the analyses described below were generally performed on a “study population” of 1,380. However analyses incorporating age were carried out on a reduced data set of only 1,333 persons, because of missing data from 47 people (Table 4.1).

During the prospective study (ca. 19 months), 163 emigrants, and 10 deaths were recorded in the study population; the whole population also received 126 immigrants and 37 births (Figure 4.2). Amongst the remaining study population, 441 persons refused to participate in the second MST application. Thus, 55,5 % (766) of the study population received a second MST (Figure 4.2).

Figure 4.1 Population Structure

Age groups

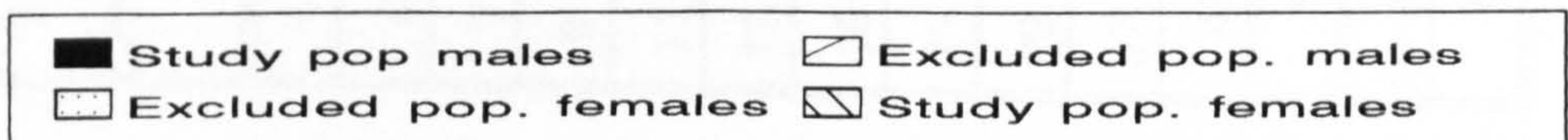
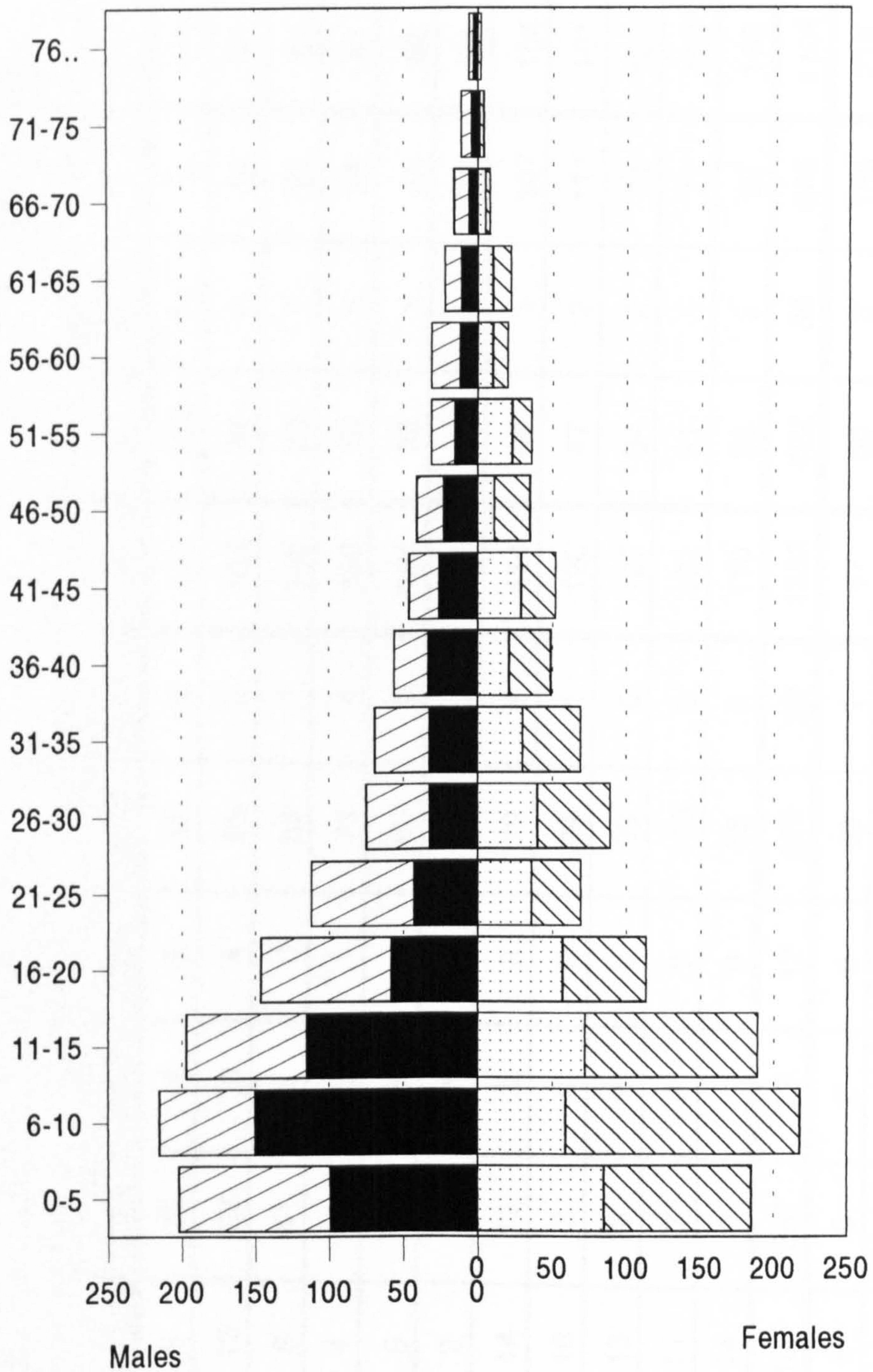


Table 4.1 Distribution of the study and excluded populations by age, clinical status and MST

AGE GROUPS	STUDY POPULATION										EXCLUDED POPULATION				TOTAL
	HEALTHY		LESIONS		SCARS		sub-total	healthy	lesions	scars	sub-total				
	mst+	mst-	mst+	mst-	mst+	mst-									
1 - 3	5	78	13	3	12	2	113	101	11	8	120	233			
4 - 6	12	62	10	4	65	3	156	44	5	28	77	234			
7 - 8	9	41	6	0	53	7	116	20	2	29	51	167			
9 - 10	4	35	6	1	73	4	123	13	3	25	41	164			
11 - 12	9	19	6	0	68	2	104	26	1	35	62	166			
13 - 15	2	24	7	2	91	3	129	24	8	62	94	222			
16 - 20	14	12	3	3	79	3	114	46	1	107	154	268			
21 - 28	16	3	6	0	94	1	120	47	3	111	161	280			
29 - 36	13	6	9	0	99	0	127	34	2	71	107	235			
37 - 48	11	7	3	0	100	0	121	31	1	77	109	230			
49+	14	0	4	0	88	4	110	36	2	92	130	240			
total	109	287	73	13	822	29	1333	422	39	645	1106	2439			
no-age	8	6	2	0	30	1	47	60	3	155	218	265			
total	117	293	75	13	852	30	1380	482	42	800	1324	2704			

TABLE 4.2 Distribution of the clinical, MST status and MST conversions by villages of the study poopulation

VILLAGES	WHOLE POP.	STUDY POPULATION									
		TOTAL	LESIONS	L+	SCARS	S+	MST +	M+	R*	C*	P*
BUENOS AIRES	182	128	14	0.11	97	0.76	111	0.87	11	4	0.36
LA SOLEDAD	397	177	20	0.11	125	0.71	154	0.87	15	6	0.4
PLAN DE ARMAS	217	91	9	0.1	44	0.48	65	0.71	12	5	0.42
LA DORADA	176	77	7	0.09	50	0.65	62	0.81	11	3	0.27
SANTA SOFIA	177	73	6	0.08	47	0.64	56	0.77	14	7	0.5
YOLANDAS	109	74	4	0.05	54	0.73	62	0.84	8	0	0
SAN PEDRO	302	190	10	0.05	109	0.57	134	0.71	32	10	0.31
TAGUAL	223	137	7	0.05	66	0.48	86	0.63	40	5	0.13
VALPARAISO	211	82	3	0.04	50	0.61	57	0.71	17	5	0.29
DELICIAS	125	95	3	0.03	59	0.62	66	0.69	26	4	0.15
CUCUCHONAL	165	103	2	0.02	73	0.71	78	0.76	13	5	0.38
MIRALINDO	420	153	3	0.02	108	0.71	113	0.74	27	8	0.3
TOTAL	2704	1380	88	0.06	882	0.64	1044	0.76	226	62	

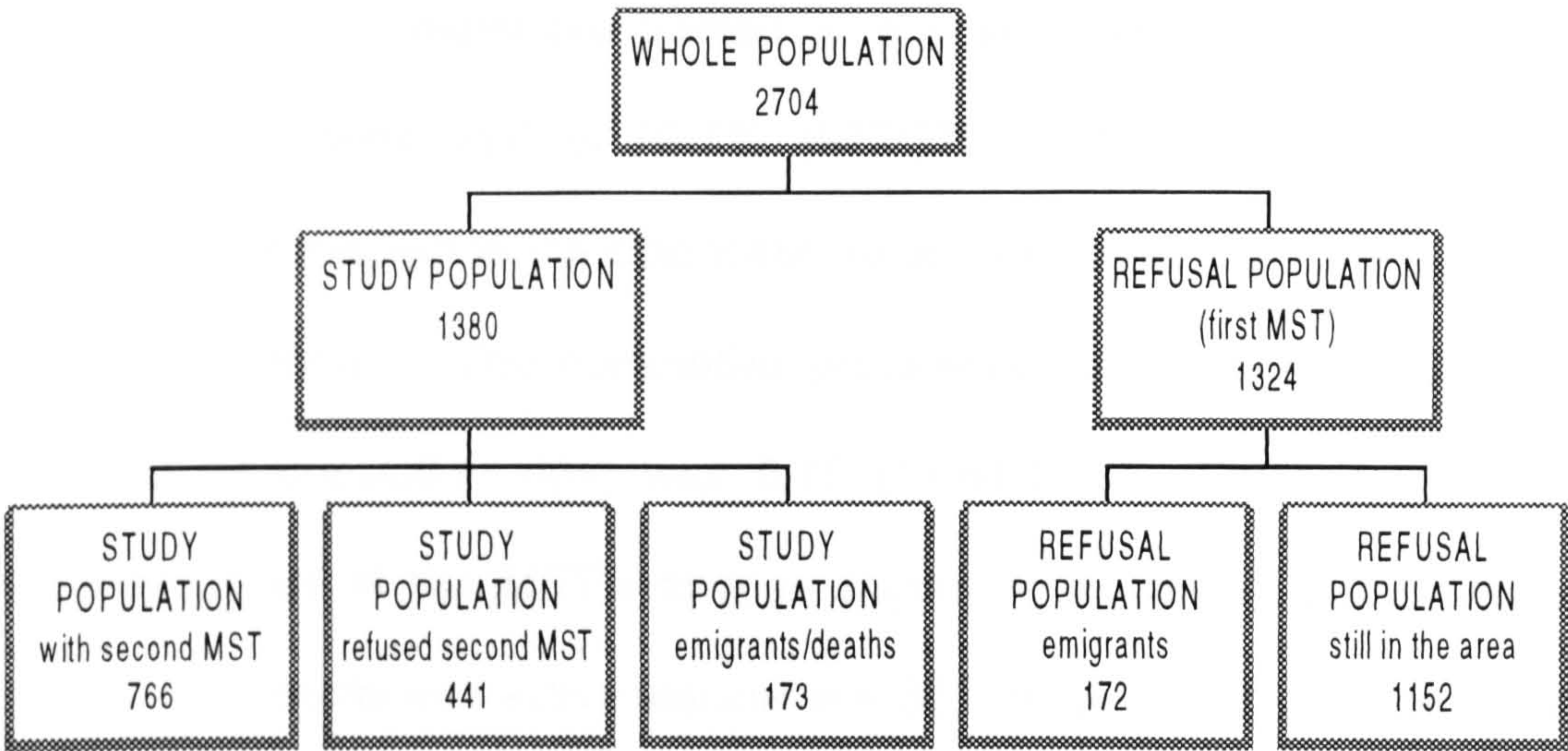
L+, S+, M+, proportion of people with lesions,scars and MST+

R* = MST negative 1995, retested in 1997

C* = number of conversions M-1995 to M+ 1997

P* = proportion of conversions

Figure 4.2 Distribution of excluded and study populations in Opon



4.2.2 Population infection rate

In the cross-sectional study (1995), MST was applied to 1,380 persons (Table 4.1). The distribution of induration sizes shows a bi-modal shape (Figure 4.3 and Figure 4.4) indicating a clear differential response between uninfected and infected people. In order to define a cut-off point for M+, the distribution of MST induration sizes was compared between L+ (scarred) and L- people (Table 4.3). It is clear from these data that there is no empirical reason for choosing the “text book” cut-off point of 5 mm, as the majority of people with induration sizes between 1 - 4 mm also had scars. In contrast, clinical cases were a small minority amongst those with a zero MST response. However, given the few data, the choice of cut-off point between 1 - 4 mm must be somewhat arbitrary. In the analysis described in this

thesis, I use a relatively conservative cut-off point of 3 mm, i.e., induration sizes of 3 mm or greater are treated as a positive MST response or "M+". This gives a "sensitivity" of 95.5% (927/970) and "specificity" of 71.4% (293/410) with respect to the diagnostic value of MST for the identification of clinical symptoms. The cumulative prevalence of infection amongst the whole study population (M+) was 0.75 (1,044/1,380) (Table 4.4). The induration sizes of the MST response according to clinical status was as follows: (1) people with active lesions: n = 88; range 0 - 26 mm; geometric mean 8.07 mm (95% C.I. 7.87-8.26 mm); (2) scarred population: n = 882; range 0 - 49 mm; geometric mean 12.19 mm (95% C.I. 12.14 - 12.23 mm); (3) "healthy" people: n = 410; range 0 - 26 mm; geometric mean 1.02 mm (95% C.I. 0.91 - 1.13).

Table 4.3 The relationship between clinical status and MST size

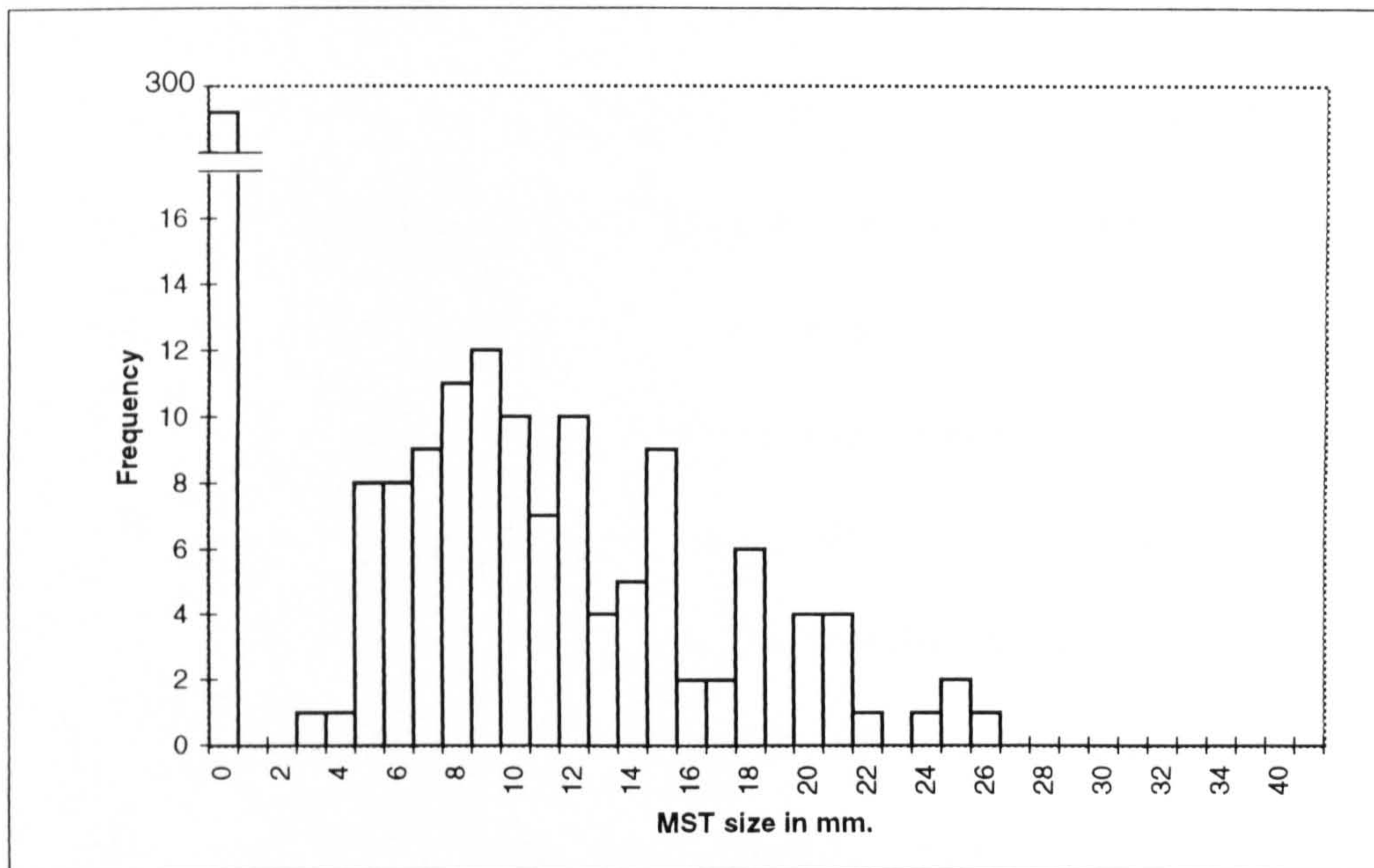
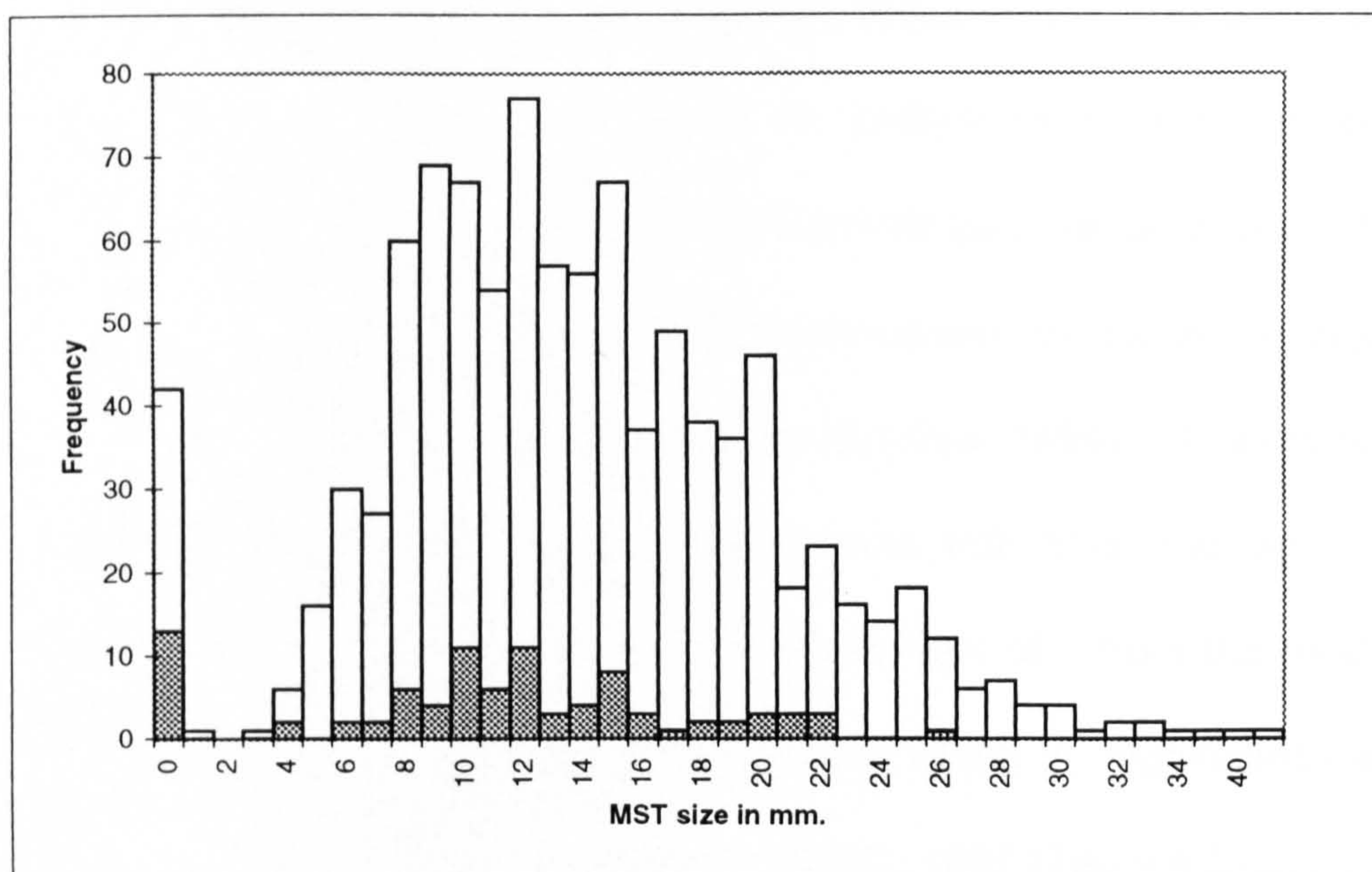
MST SIZE (mm)	FREQUENCY ACCORDING TO CLINICAL STATUS			
	HEALTHY	SCARS	TOTAL	% SCAR*
0	292	29	321	9.1
1	0	1	1	100
2	0	0	0	0
3	1	1	2	50
4	1	4	5	80
5	8	16	24	66.7
6	8	28	36	77.7
7	9	25	34	73.5
8	11	54	65	83
9	12	65	77	84.4
10	10	59	69	84.8
> 11	58	600	656	91.1

* percentage of people with scar for each MST size

Table 4.4 Distribution of the study population by clinical and MST status including follow up

CLINICAL AND MST STATUS 1995			CLINICAL AND MST STATUS 1997						
clinical status	MST status	n	TOTAL RE-TESTED	NEW LESIONS			NO NEW LESIONS		
				M+	M-	NT	M+	M-	NT
ACTIVE LESIONS	M+	75 (36/58)*	50	1 (1/1)	0	2 (0/0)	49	0	23
	M-	13 (2/6)	6	0	0	0	4	2	7
SCARS	M+	852	439	16 (6/14)	0	4 (1/2)	414	9	409
	M-	30	15	1 (1/1)	1 (1/1)	0	5	8	15
HEALTHY	M+	117	51	5 (0/2)	0	3 (1/1)	39	7	63
	M-	293	205	36 (27/31)	8 (2/5)	5 (3/3)	16	145	83
TOTAL		1380	766	59 (35/49)	9 (3/6)	14 (5/6)	527	171	600

* A (B / C). A = total number of suspected cases detected in surveys; B = number of cases with diagnosis and clinical history; C = total number of cases with clinical history

Figure 4.3 Frequency distribution of MST induration sizes amongst L-**Figure 4.4** Frequency distribution of MST induration sizes amongst L+

Filled bars represents patients with active lesions; open bars scarred population

Between June 1995 and March 1997, 766 people were MST tested in two sample rounds. For the calculation of incidence rates, the mean number person-months at risk between the first and second MST date was 16 months. Amongst the 226 M- people who were re-tested, 62 converted to M+, resulting in an incidence of 0.21 (95% C.I. 0.17-0.24) conversions/person-year. Evidence of past infections were detected in 10 people (6 scars, 4 lesions) who converted from M-₁₉₉₅ to M+₁₉₉₇ (Table 4.4), suggesting that these conversions reflect infections already prevalent in 1995 but with an extended incubation period prior to MST conversion, rather than incident infections during the prospective study. Thus, our most reliable assessment of incidence rate is 0.19 (95% C.I. 0.15 - 0.23) conversions/person-year which is calculated from the 52 M-L-₁₉₉₅ who converted during the prospective study. This measure of incidence represents transmission during the period of the prospective survey. In order to determine if this incidence rate is characteristic of recent years in the Opon focus, the force of infection (λ) was calculated by fitting a simple infection-recovery model to the cross-sectional age prevalence data for M+ (Williams and Dye, 1994). The model makes the assumption of constant transmission rate with time and age. The latter assumption is validated below. The estimate of λ from the model was 0.13 (95% C.I. 0.12-0.14) cases/person-years, which is significantly smaller than the incidence rate detected between 1995 -1997 (Table 4.5).

4.2.3 Population clinical infection rate

In 1995, the cumulative prevalence of clinical detected leishmaniasis (i.e. the proportion of the population with scars or lesions), L^+_{1995} , was 0.72 (95% C.I. 0.69 - 0.74) cases/person [970/1,380] with a scar prevalence of 0.64 (95% C.I. 0.61 - 0.66) cases/person [882/1,380] and an active prevalence (lesions) of 0.063 (95% C.I. 0.047-0.075) cases/person [88/1,380] (Table 4.4). During the follow up, 61 patients with new lesions were enrolled in the study, giving an overall clinical incidence rate of 0.030 (95% C.I. 0.019 - 0.041) cases/person-years. However, 19 of these patients had previous scars ($n = 18$) or lesions ($n = 1$), suggesting that their new lesions could represent either a reactivation of previous infections or an extension of chronic infections, respectively (Table 4.4).

Hence, the most reliable estimate of the clinical incidence rate in the study population is 0.1 (95% C.I. 0.085 - 0.11) cases/person-years, calculated from the 410 people who had no history of clinical leishmaniasis prior to 1995. As before, λ was calculated by fitting an infection recovery model to the age prevalence curves for L^+ (Table 4.6). In this case, clinical incidence calculated from the follow up, was not significantly different than the fitted by force of infection the model to the cross-sectional data: $\lambda = 0.14$ (95% C.I. 0.11-0.16) cases/person-years.

**TABLE 4.5 . Force of infection and Recovery rate calculated from
MST age prevalence data**

AGE (years)	TOTAL POPULAT.		FEMALES		MALES	
	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE
1-3	113	30	50	12	63	18
4-6	156	87	79	43	77	44
7-8	116	68	58	31	58	37
9-10	123	83	72	48	51	35
11-12	104	83	55	44	49	39
13-15	129	100	62	48	67	52
16-20	114	96	56	41	58	55
21-28	120	116	58	56	62	60
29-36	127	121	68	65	59	56
37-48	121	114	57	55	64	59
49more	110	106	52	49	58	57
Total	1333	1004	667	492	666	512
	λ	ρ	λ	ρ	λ	ρ
Estimate	0.135	0.006	0.123	0.005	0.148	0.006
SD	7.00E-03	0.002	0.009	0.002	0.011	0.003

**Table 4.6 . Force of infection and Recovery rate calculated from
clinical status age prevalence data**

AGE (years)	TOTAL POPULAT.		FEMALES		MALES	
	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE
1-3	113	30	50	12	63	18
4-6	156	82	79	44	77	38
7-8	116	66	58	32	58	34
9-10	123	84	72	49	51	35
11-12	104	76	55	38	49	38
13-15	129	103	62	50	67	53
16-20	114	88	56	42	58	46
21-28	120	101	58	47	62	54
29-36	127	108	68	58	59	50
37-48	121	103	57	49	64	54
49more	110	96	52	49	58	47
Total	1333	937	667	470	666	467
	λ	ρ	λ	ρ	λ	ρ
Estimate	0.147	0.025	0.139	0.022	0.154	0.028
SD	0.011	0.005	0.015	0.006	0.016	0.007

The proportion of people infected in a determined age "a" was calculated by maximum likelihood (Williams and Dye, 1994) with the following equation:

$$p(a) = \frac{\lambda}{\lambda + \rho} (1 - e^{-(\lambda + \rho)t})$$

λ = Force of infection
 ρ = Recovery rate

(see Materials and Methods)

4.2.4 Personal risk factors for infection

4.2.4.1 Gender

The cumulative prevalence of infection by gender was slightly higher for males, 0.77 (95% C.I. 0.73 - 0.80) cases/person [535/692], than for females, 0.73 (95% C.I. 0.69-0.76) cases/person [509/688]; but there was no significant gender difference ($\chi^2 = 2.08$, 1 D.F., $P = 0.149$).

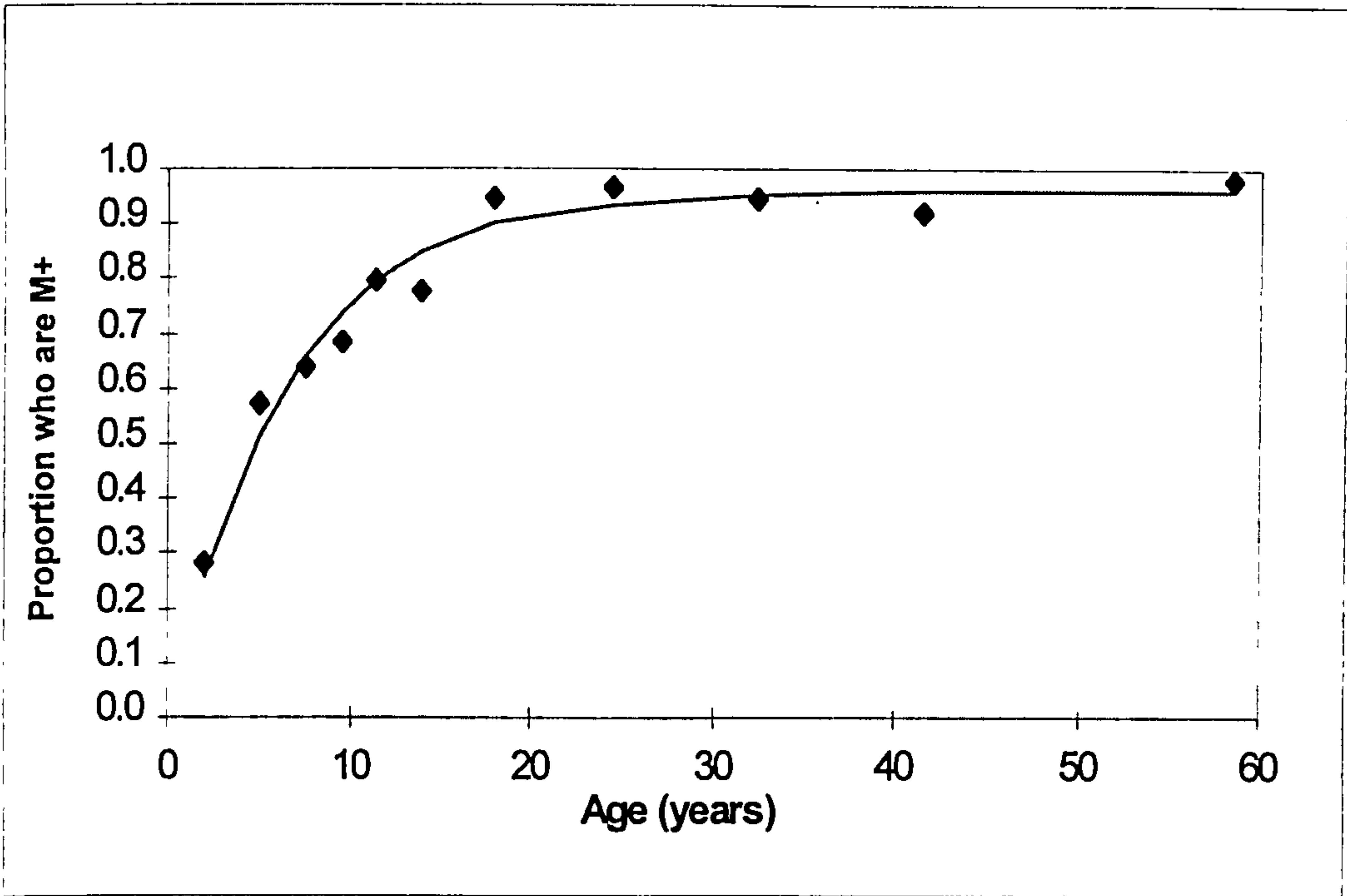
In the follow up study, incidence of infection in males, 0.10 (95% C.I. 0.05 - 0.14) cases/person-years, was also not significantly different from the incidence of infection in females, 0.13 (95% C.I. 0.07- 0.18) cases/person-years. Similarly, λ calculated for males from the age prevalence curves for M+ (Table 4.5) was 0.14 (95% C.I. 0.11 - 0.16) cases/person-years (Figure 4.5a), which was not significantly different from λ calculated for females: 0.12 (95% C.I. 0.10 - 0.13) cases/person-years (Figure 4.5b). Thus there is no evidence of any difference in the risk of infection between males and females.

4.2.4.2 Age

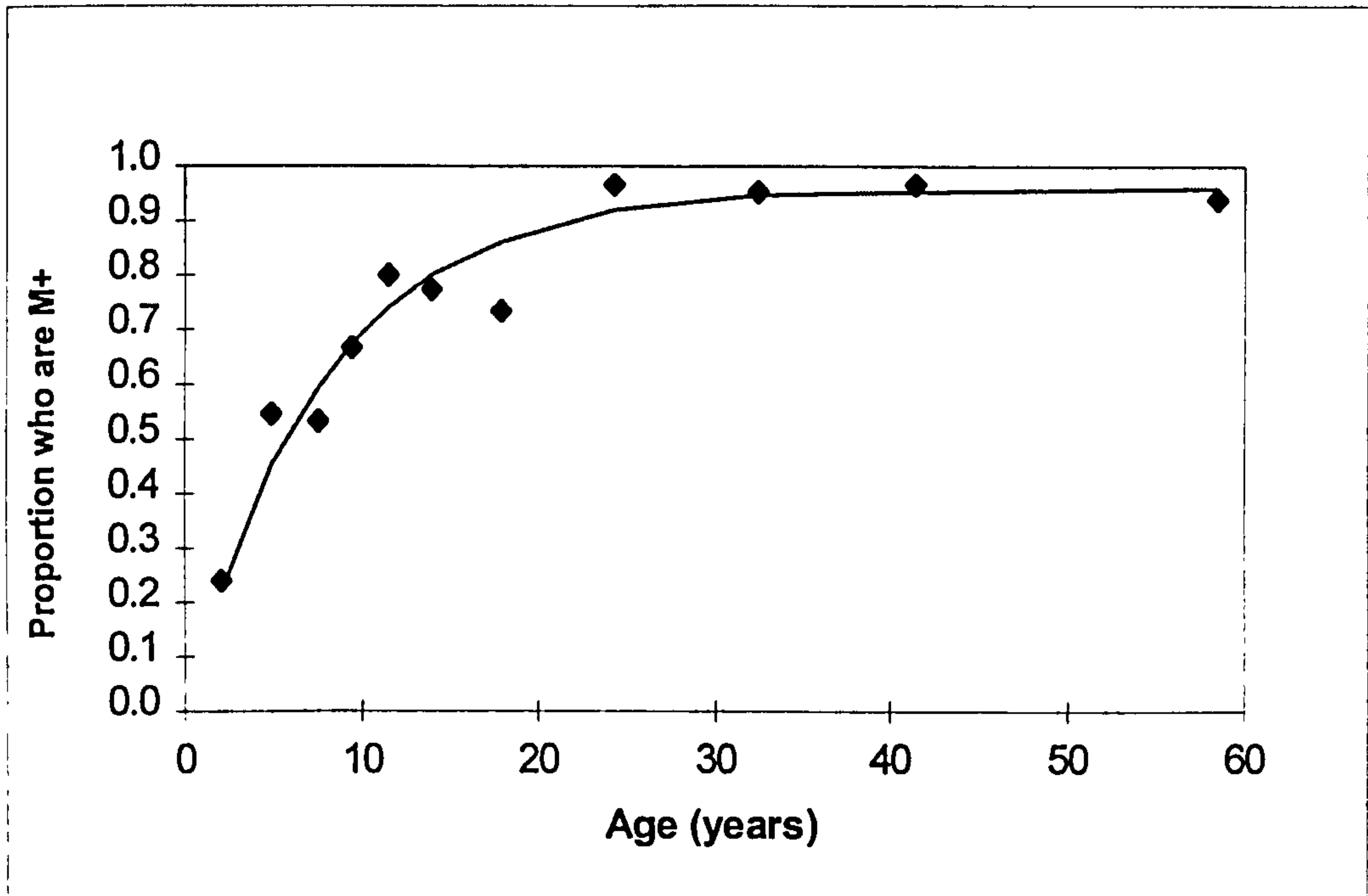
Because of the relatively high transmission rate in the Opon focus, the majority of M-₁₉₉₅ (224/336, 0.66) are concentrated amongst children less

Figure 4.5 Age prevalence curves of infection by gender

a) Age prevalence curves of infection for males



b) Age prevalence curves of infection for females

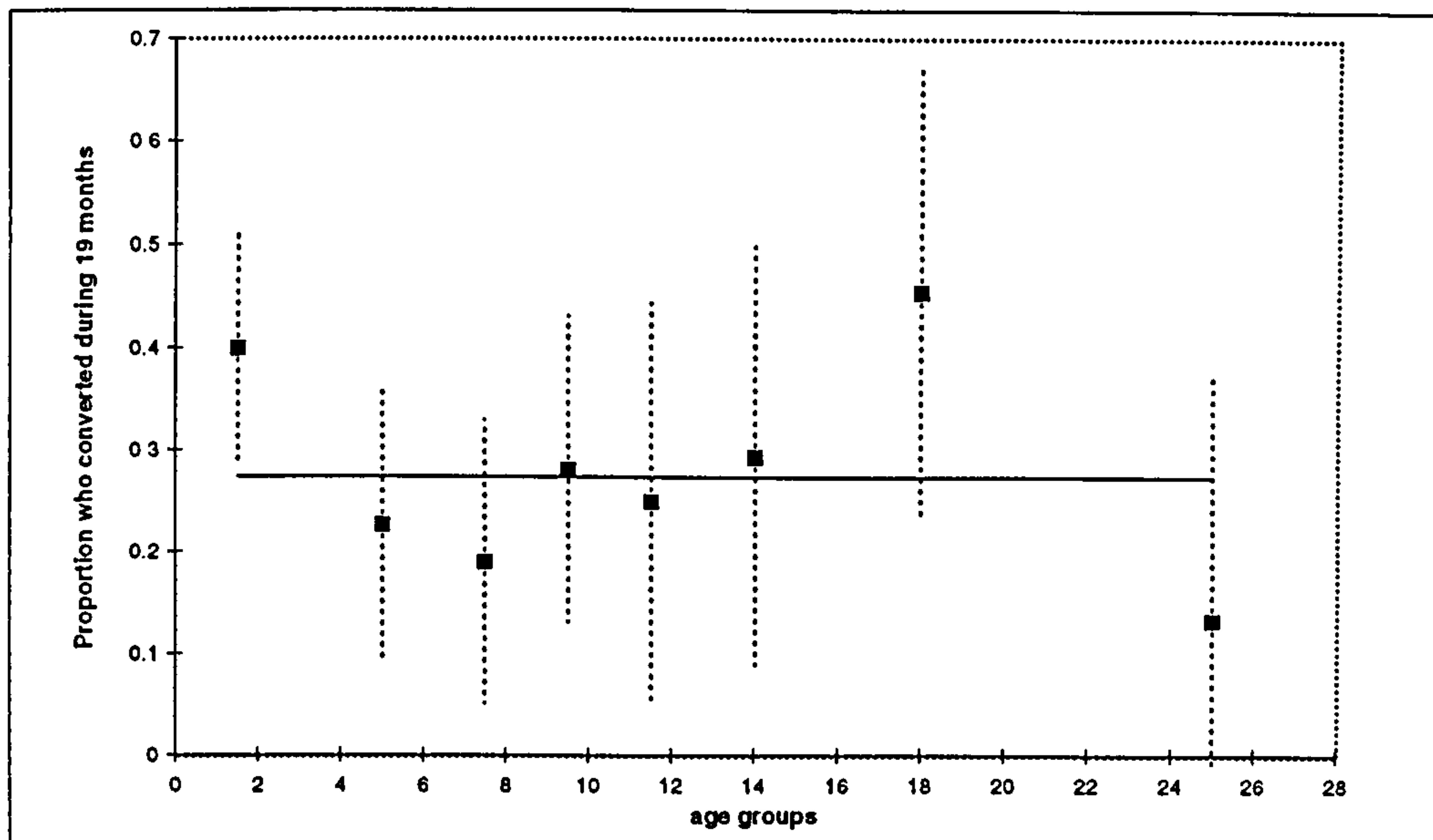


Age prevalence curves for M+ detected during the cross-sectional study. Each point represents the proportion m+ within a particular age group. Lines were drawn by maximum likelihood fit to the infection-recovery model (Williams, 1994, see Table 4.5)

than 10 years old (Table 4.5 and Table 4.6). This explains the relatively low mean age of 8.58 (95% C.I. 6.68 - 10.47) years amongst the 62 people who converted during the study. However, there was no significant difference between the mean age of the people who remained M- in 1997, 10.15 (95% C.I. 8.40-11.89) years, compared to the mean age of “converters” ($\chi^2 = 1.73$, $P > 0.05$, 1 D.F.). The absence of any relationship between age and infection rate (i.e. exposure to sandfly vector) is illustrated in Figure 4.6, which demonstrates that there is no trend between the incidence rates calculated for eight age-groups (with equal denominators) (ANOVA, $r^2=0.008$; $F = 0.05$; $P > 0.05$) (Figure 4.6, and Table 4.7). Thus, new infections occurred irrespective of age, validating the key assumption made in the infection-recovery model to calculate λ from age prevalence data (see Section 4.2.2)

Table 4.7 The MST conversion rate by age

AGE	Number of M- /95	Number re-tested /97	Number converted	proportion	standard errors
1-3	83	45	18	0.4	0.12
4-6	69	44	10	0.23	0.13
7-8	48	42	8	0.19	0.14
9-10	40	32	9	0.28	0.15
11-12	21	20	5	0.25	0.19
13-15	29	17	5	0.29	0.2
16-20	18	11	5	0.46	0.22
21-more	21	15	2	0.13	0.24
TOTAL	329	226	62	0.274	

Figure 4.6 MST conversion rate by age

Conversion rate by age. Squares are the proportions converting in each age class over the 19 months period, and dotted line are the standard errors. The solid line is the average conversion rate.

4.2.4.3 Clinical status.

Conversions were detected in 47% of people with previous lesions (10/21; 95% C.I. 31.7 - 62.2%) and in 25% of the healthy people (52/205; 95% C.I. 19 - 30%) re-tested in 1997. The difference between the two groups was statistically significant ($\chi^2 = 4.72$, $P < 0.05$; Odds Ratio = 2.67, 95% C.I. 0.95 - 7.36). Thus, in the univariate analyses, clinical status was the only significant explanatory factor identified for predicting the individual risk of MST conversion: there was no relationship between individual risk of conversion and age, gender or village. This result was confirmed by a multivariate

analysis in which clinical status was the only variable retained in the minimal adequate model ($\chi^2 = 6.826$, 2 D.F., $P < 0.05$, $r^2 = 0.025$). The relatively high conversion rate in people with prior clinical infections is, presumably, because of their persistent but intermittent infection status, rather than due to reinfections during the prospective survey.

4.2.5 Household risk factors for infection

A total of 114 houses in the study area were randomly selected (Table 4.8) and the surrounding vegetation was classified (see Chapter 5). Five types of vegetation were defined as potential risk factors for domiciliary transmission: primary forest, secondary forest, cacao, pasture and other crops (fruit crops and sugar cane). The relative abundance (in percentage) of this vegetation was calculated within concentric circles with radiuses from the house of 50 m, 100 m, 200 m, 300 m, and 800 m, respectively. These values were then tested in a multivariate analysis for their explanatory power to predict either the prevalence or incidence of infection within each household. Given the large number of variables and models tested, a relatively stringent requirement of $P < 0.01$ was chosen as the indicator of significance.

In total, 10 different models were analysed but only one produced a minimal adequate model with a significant explanatory variable. In this model, the outcome variable was the proportion of MST positives in a house, and the explanatory variable was the percentage of pasture surrounding a house up to radius of 800 m. There was a significant negative relationship ($\chi^2 = 10.36$, $P < 0.005$) between these two variables as shown in Figure 4.7, but the strength of the association was very low: $r^2 = 0.05$ (Table 4.9). i.e., only 5% of the variance in house prevalence was explained by variation in the relative cover of pasture around house.

Table 4.8 The number of houses in each village with MST survey and vegetation coverage data

VILLAGE	HOUSES WITH MST POP. DATA	HOUSES WITH VEGETATION DATA
Buenos Aires	30	10 (1*)
Cucuchonal	19	10
Delicias	19	10
La Dorada	18	9
La Soledad	49	21
Miralindo	40	9
Plan de Armas	27	9
Santa Sofia	14	9 (2*)
San Pedro	45	10
Tagual	35	10
Valparaiso	19	0
Yolandas	16	10
TOTAL	331	117

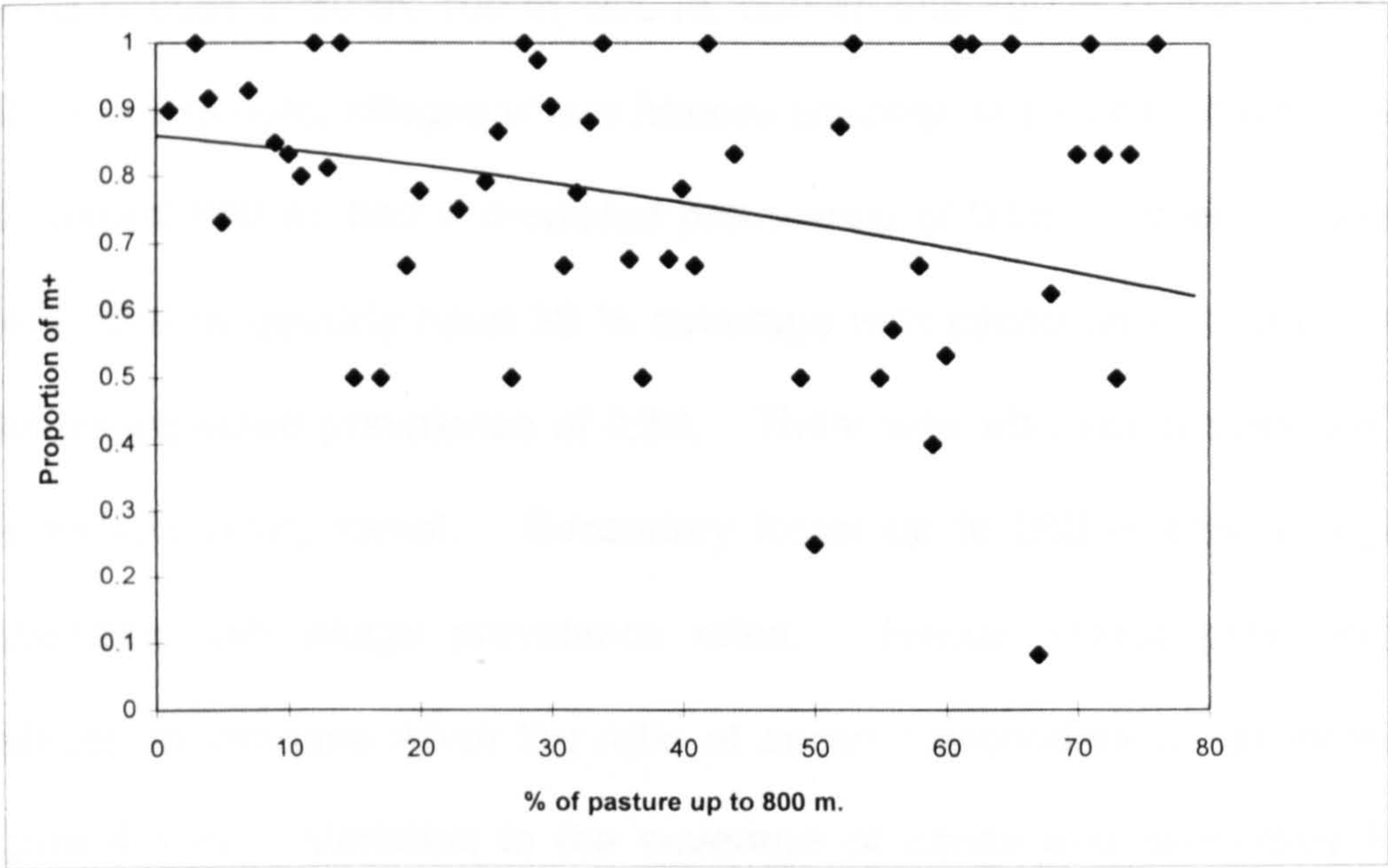
* # of houses without MST data

Table 4.9 Household and village risk factors for infection: Vegetation type.

	MODEL	50 m.	100 m.	200 m.	300 m.	800 m.
CONVERSIONS BY VILLAGE	intercept (s.e.)	-	-	1.197* (1.11)	1.85** (1.03)	0.78** (0.69)
	secondary forest	-	-	-0.08 (0.04)	-0.10 (0.03)	-0.06 (0.02)
	r ²	-	-	0.205	0.382	0.37
PREVALENCE BY VILLAGE	intercept (s.e.)	0.71*** (0.13)	0.65*** (0.13)	2.46*** (0.57)	0.57*** (0.12)	0.33*** (0.15)
	cacao	0.02 (0.005)	0.028 (0.006)	0.022 (0.006)	0.035 (0.006)	0.056 (0.009)
	secondary forest			-0.06 (0.02)		
	r ²	0.295	0.382	0.872	0.686	0.788
CONV./HOUSE		-	-	-	-	-
PREVALENCE BY HOUSE	intercept (s.e.)	-	-	-	-	1.82** (0.22)
	pasture	-	-	-	-	-0.016 (0.005)
	r ²	-	-	-	-	0.05

* P < 0.05 / ** P < 0.01 / *** P < 0.001. Estimates (standard errors) and significance of explanatory variables for village/houses incidence and cumulative prevalence, by multiple logistic regression (degrees of freedom = 10 for incidence and prevalence per village, 113 for prevalence per house).

Figure 4.7 Household risk factors for infection: the relationship between the extent of pasture land surrounding a house and the proportion of m+



The line represents fitted values (see Table 4.9) and diamonds the observed values.

4.2.6 Village risk factors for infection

The proportion of M+ varied from 0.63 in the village of Tagual to 0.87 in the village of Buenos Aires (Table 4.2). In this section, whether any of the inter-village variation in transmission rate can be explained by inter-village variation in vegetation coverage (i.e. land use) is tested. A measure of vegetation coverage for each village was calculated by averaging the data from a maximum of 10 houses/village in each of the 11 villages tested (Table 4.8). A multivariate analysis were carried out using five different village measurements, according to the vegetation dataset chosen (50 m, 100 m, 200 m, 300 m, and 800 m). Given the large number of models and variables, factors were only retained in the minimal adequate model when $P < 0.01$.

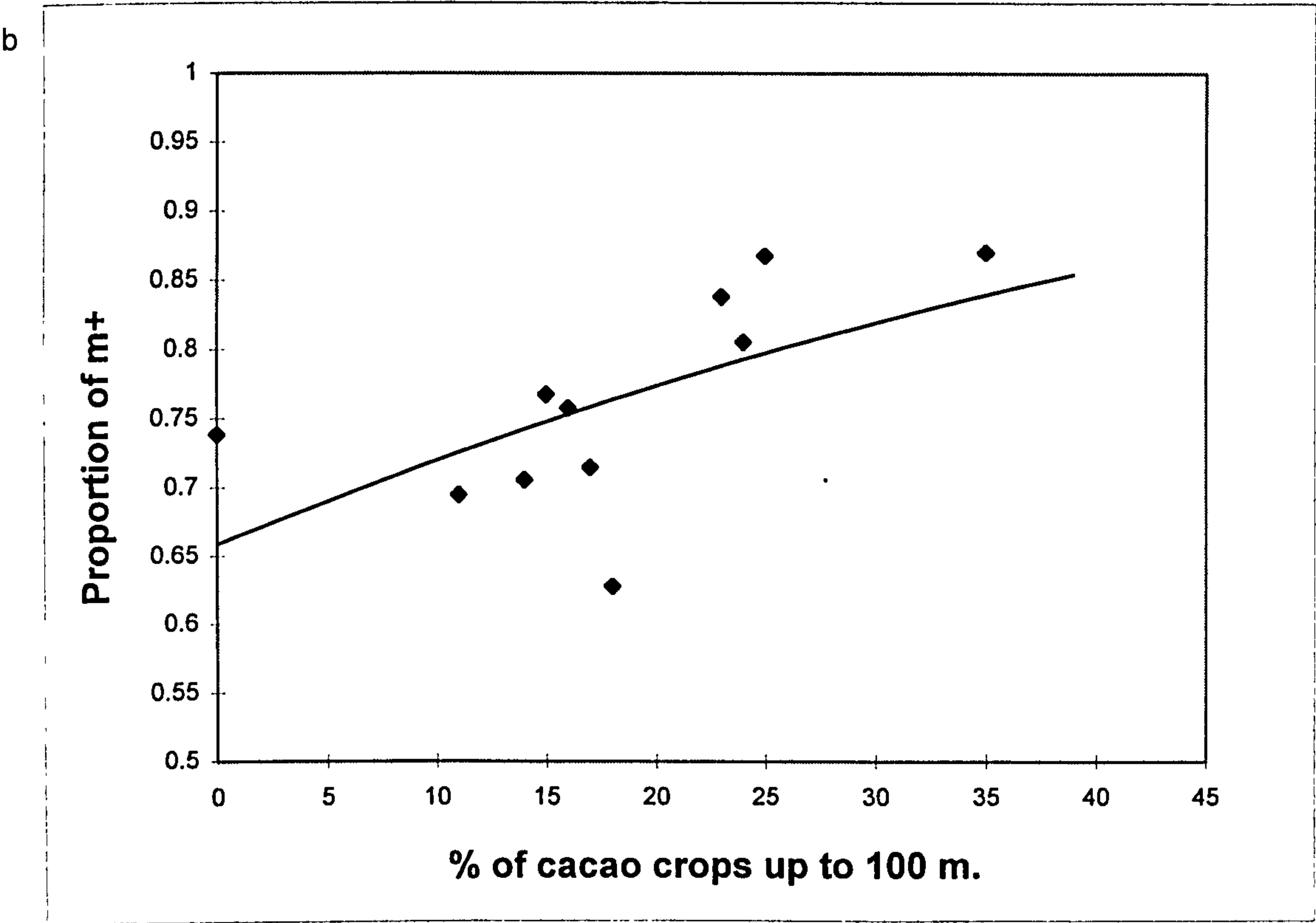
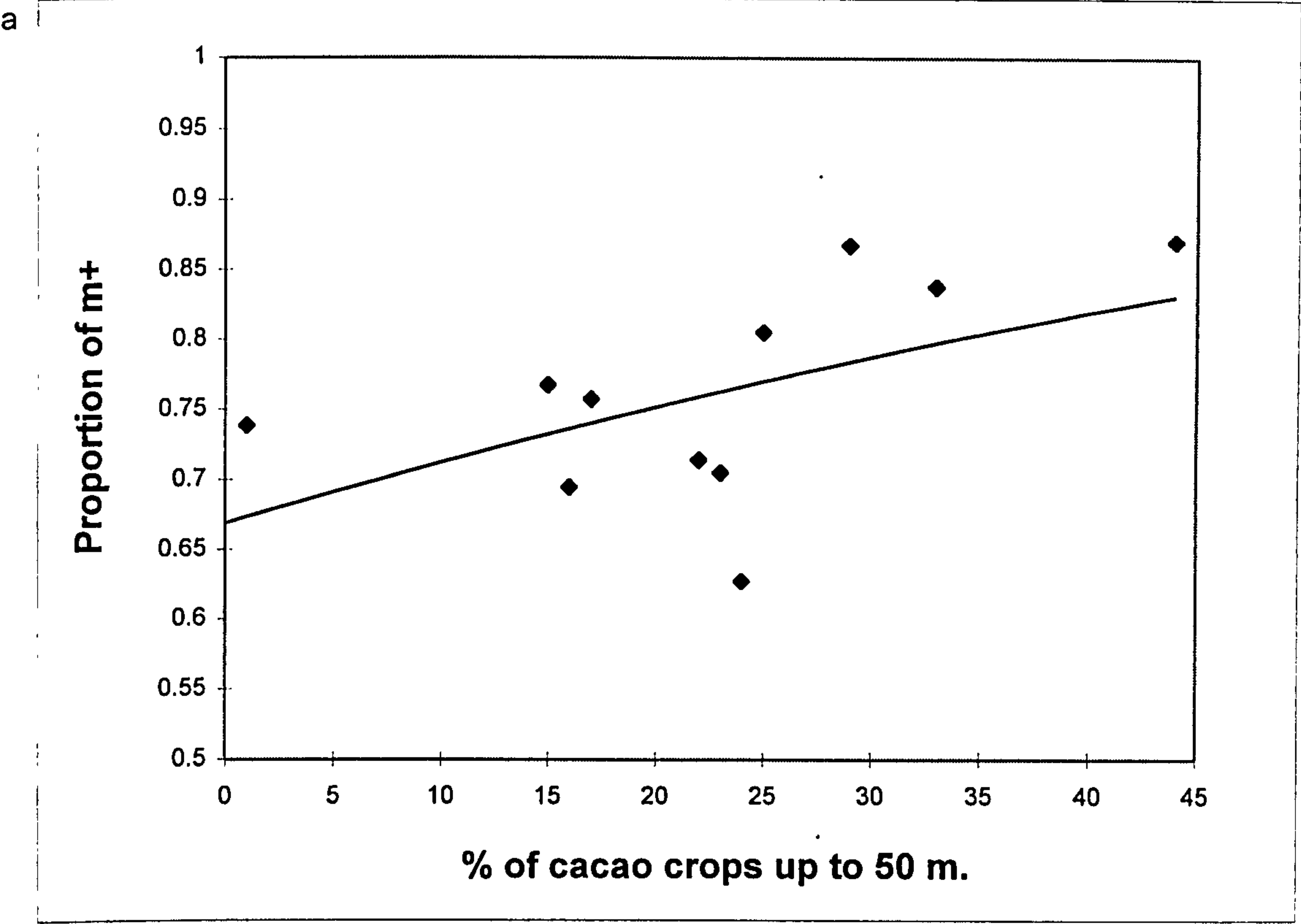
Prevalence of infection by village correlated positively with cacao crops around houses at 50 m, 100 m, 200 m, 300 m, and 800 m (Table 4.9, Figure 4.8). For example, villages where houses typically had no cacao crops within the nearest 800 m. had a predicted prevalence of 0.58. Whereas, villages where houses typically have 33 % coverage with cacao up to 800 m. would have an expected prevalence of 0.89. There was also some evidence of a role for secondary forest. Secondary forest up to 200 m had a negative association with village prevalence rates. Hence, village prevalence is predicted to increase when the ratio of cacao : secondary forest increases (Figure 4.8 c). Variation in the coverage of cacao and secondary forest

within 200 m. of houses explains a massive 87.2% of the variance in village prevalence (Table 4.9).

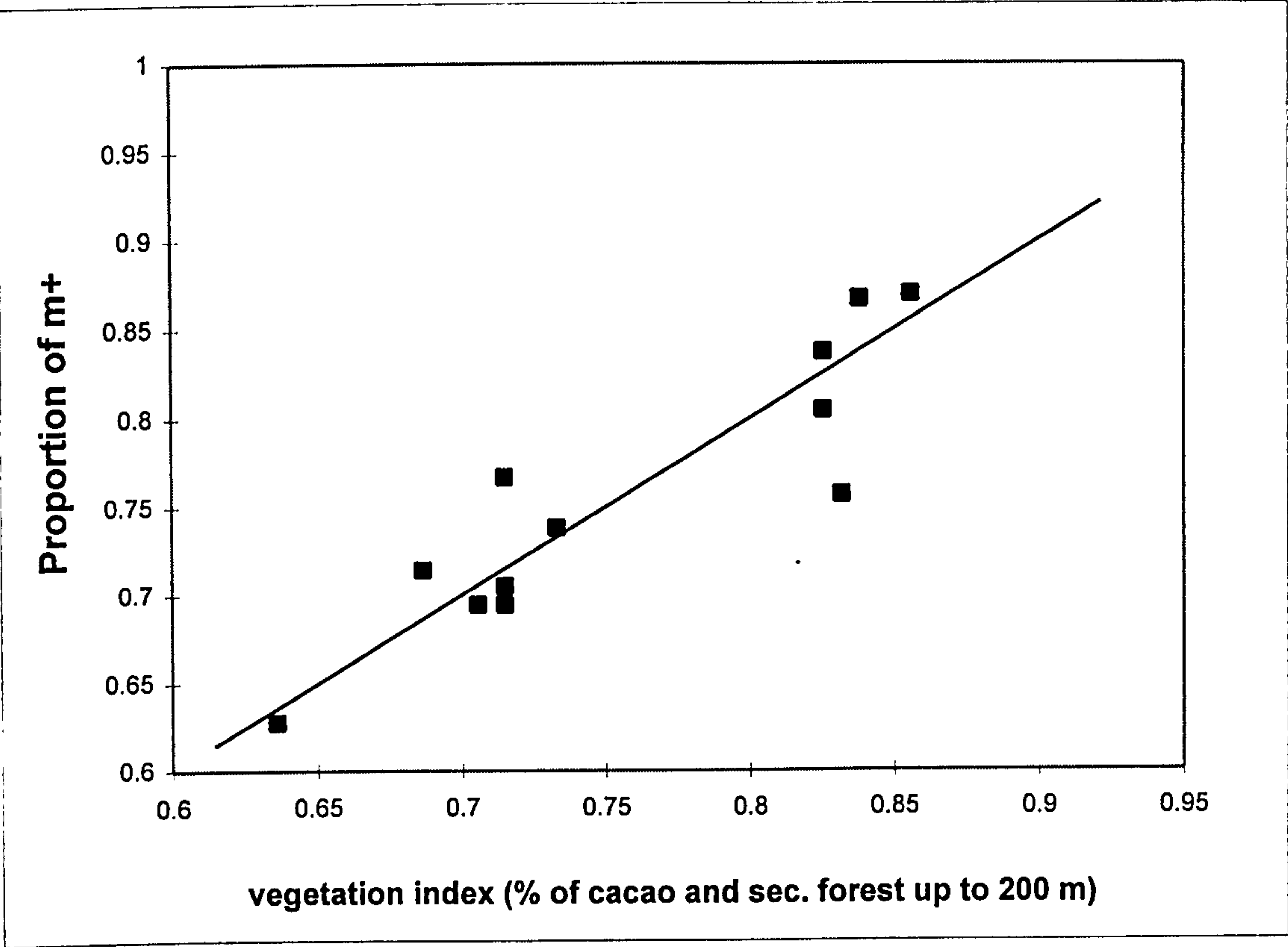
A further five multivariate analysis were carried out to test the explanatory power of the same vegetation indices to predict inter-village variation in incidence rate. Village incidence rate varies from 0.31 (95% C.I. 0.06 - 0.55) cases/person-years in the village of Santa Sofia to 0 cases/person-years in the village of Yolandas. For three out of the five models (using the mean data for 200, 300 and 800m. radiuses) a significant negative regression was detected between the coverage of secondary forest and village incidence rate (Table 4.9). The best fitting model (up to 300 m.) explained 38.2% of the variance in incidence rate.

The relationship between secondary forest and village incidence rate is illustrated in Figure 4.9. For example, Figure 4.9 c shows that in villages where houses have no secondary forest up to 800 m., the model predicts an incidence rate of 0.68/person/year compared to 0.10/person/year in villages where houses typically have 50% coverage with secondary forest within a radius of 800 m.

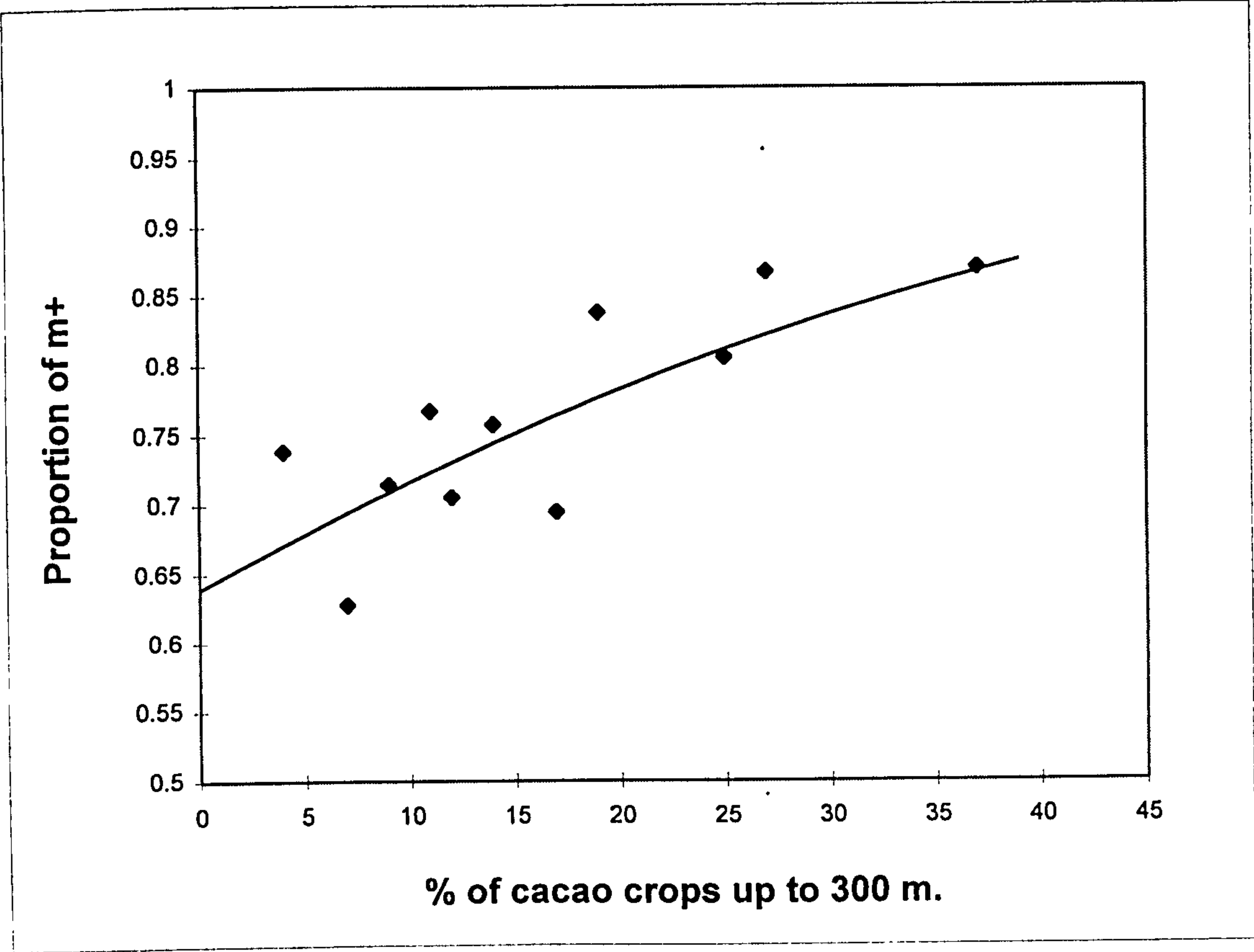
Figure 4.8 Village risk factors for infection: the relationship between vegetation coverage and cumulative prevalence in 11 villages

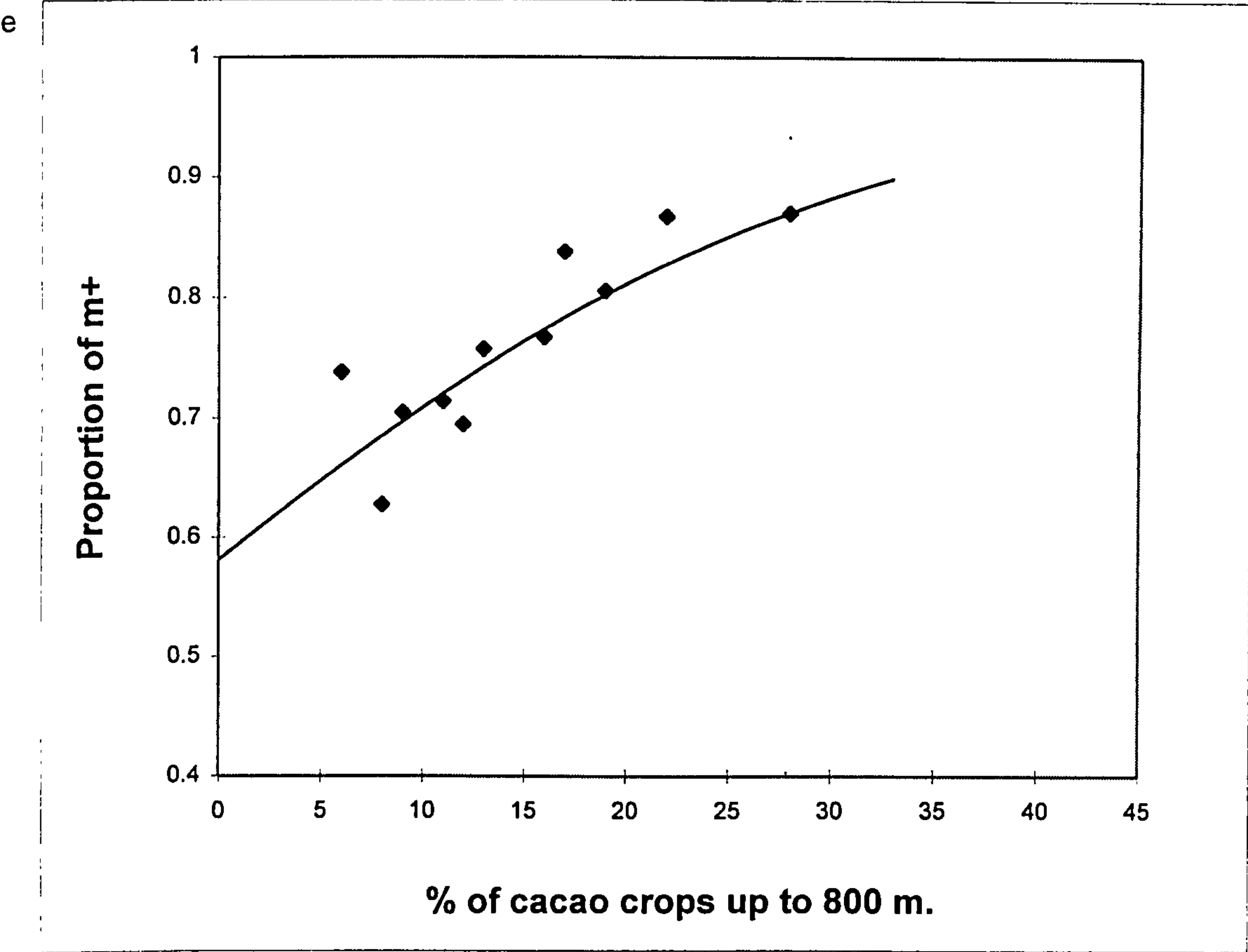


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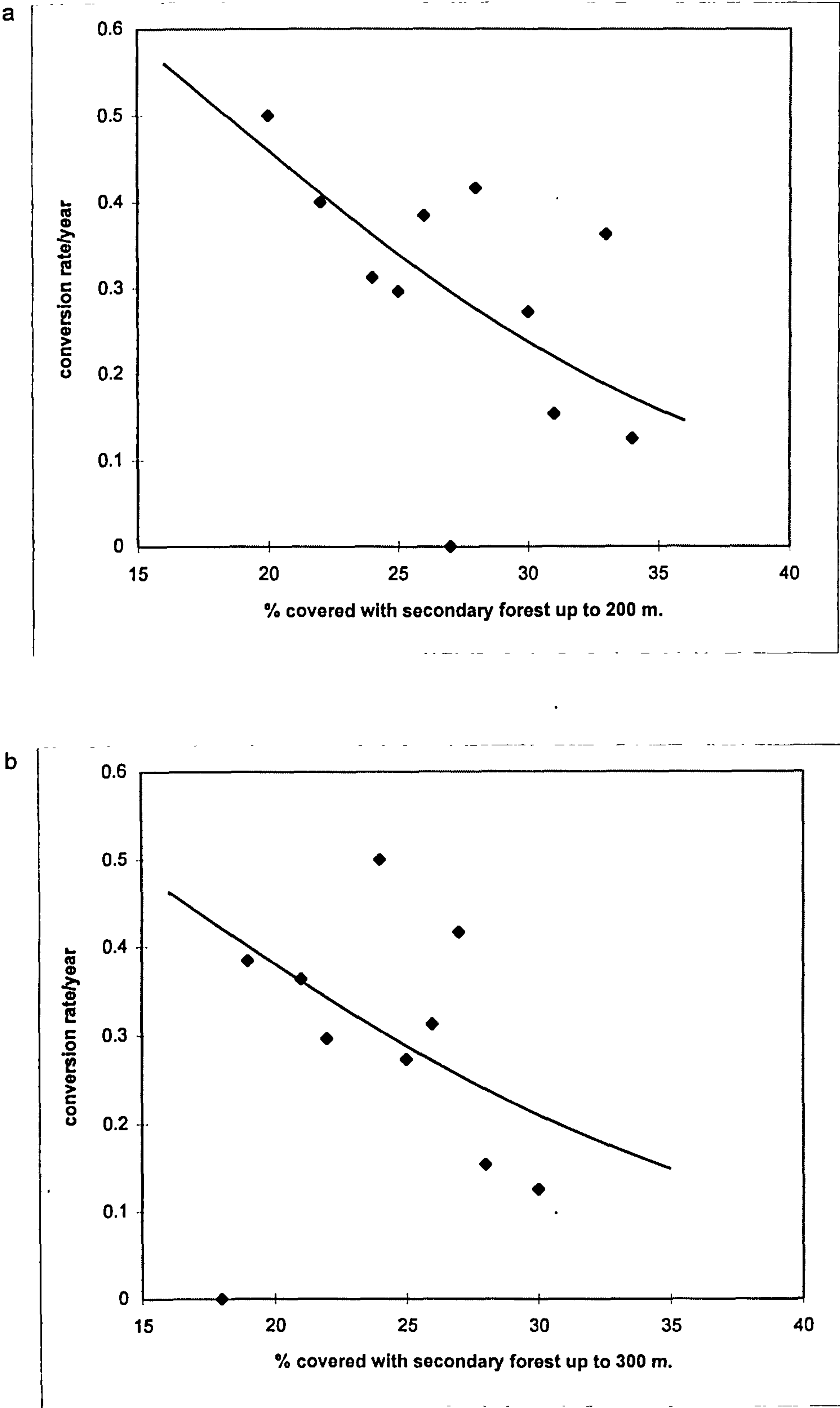
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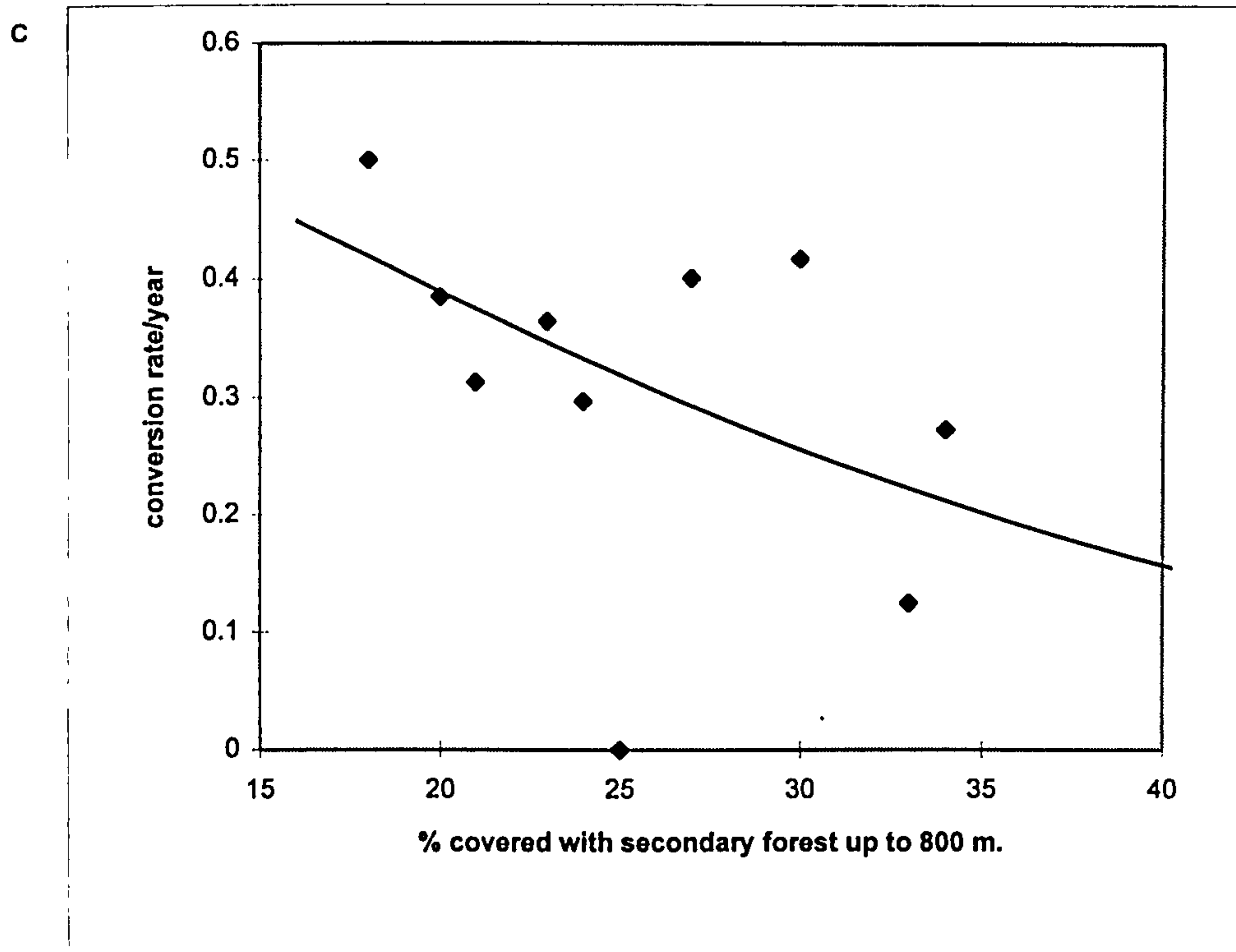




The diamonds represent the observed prevalence, the line the fitted prevalence, predicted by multiple logistic regression. Vegetation coverage measured from 50 m up to 800 m in 14 houses.

Figure 4.9 Village risk factors for infection: the relationship between vegetation coverage and incidence of infection in 11 villages





The diamonds represent the observed incidence rates, the line the expected incidence fitted by multiple logistic regression. Vegetation coverage measured from 50 up to 800 m in 14 houses

4.2.7 Risk factors for clinical symptoms with infection.

In this section, I focus on the relative risk of putative subclinical infections (MST conversion with no lesion) versus the risk of clinical infections. The “relative pathogenicity” (R) of a parasite population has been defined (Weigle et al., 1993) as the proportion of the $m+$ population with either scars or lesions ($M+L+/M+$). Using this definition, R in the Opon focus was 0.88 (927/1044) (Table 4.4). This proportion will depend not only on the relative frequency of cryptic infections, but also on the relative frequency of (1) cross reactions and (2) failure to detect past clinical infections (i.e. scars).

It is not clear which of these factors explains why the geometric mean induration size for M+L- people, 10.61 mm (95% C.I. 10.53 - 10.68 mm), was significantly smaller than the mean response in M+L+ people: 13.38 mm (95% C.I. 13.37 - 13.40 mm; t-test on log-transformed data: $t = 5.53$; 964 D.F.; $P < 0.0001$).

A more reliable estimate of the proportion of infections leading to clinical disease comes from the prospective data. The clinical infection rate amongst the M- population re-tested in 1997 was 0.13 (95% C.I. 0.083 - 0.17) cases/person-years; the subclinical infection rate was 0.09 (95% C.I. 0.05 - 0.12) cases/person-years. Thus, the proportion of MST conversions which lead to disease (α) was 0.69 (95% C.I. 0.56 - 0.82) [36/52]. Again, the possibility that some of the "subclinical" MST conversions were cross-reactions cannot be discounted. However, no significant difference was detected in the induration sizes of the MST response following "subclinical" infections (6.72 mm; 95% C.I. 5.26 - 8.59 mm) compared to the response following clinical incident infections (6.49 mm; 95% C.I. 5.84 - 7.20 mm) (t-test on log transformed data: $t = 0.29$; 51 D.F.; $P = 0.76$).

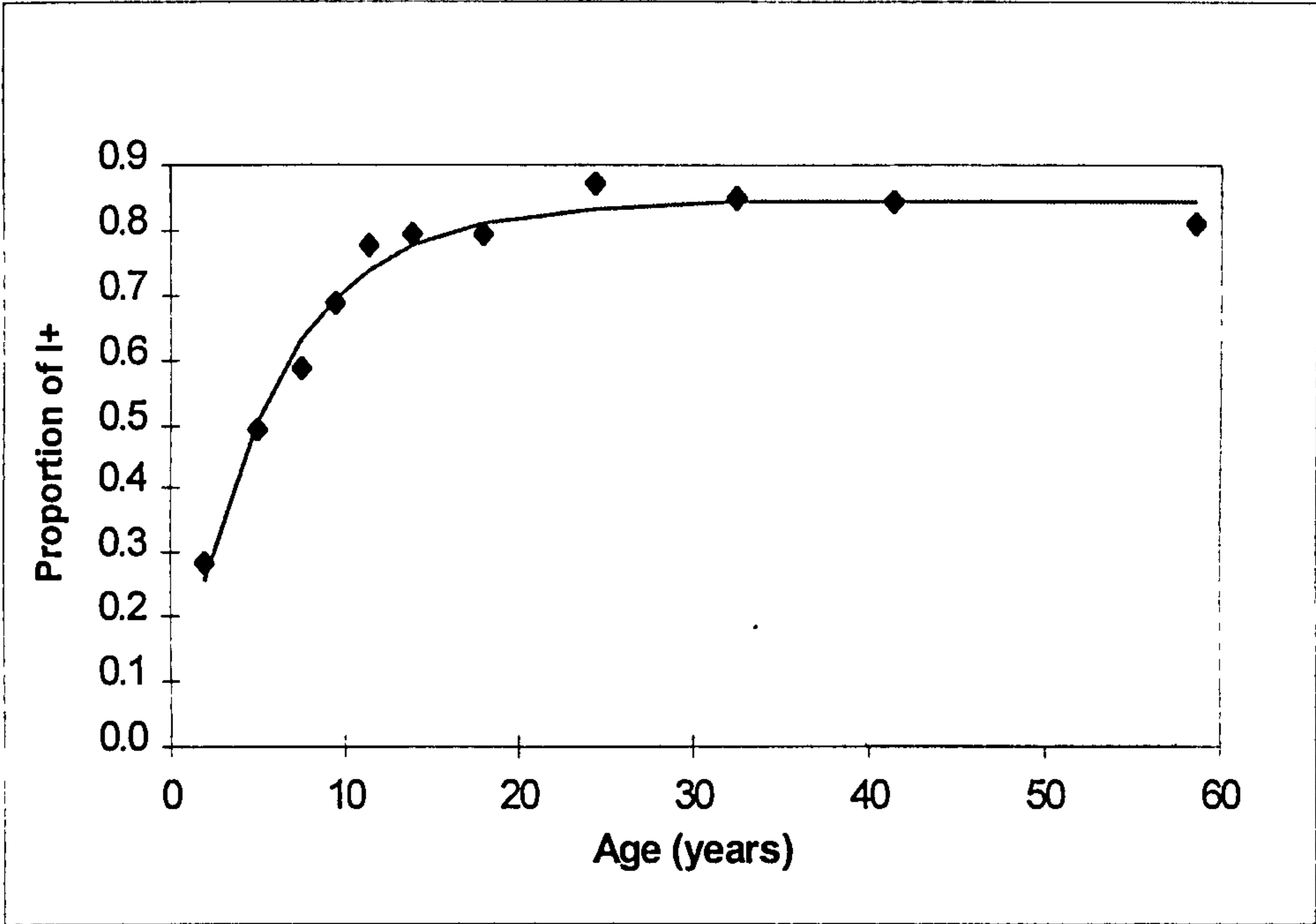
The personal risk factors which determine whether an infection leads to subclinical infections could include: (1) variability in susceptibility (e.g. genetic, nutritional or acquired immunity), (2) age, and (3) gender. The significance of these variables can be tested by comparing the two sub-

populations of people with incident subclinical (SC) or clinical (C) infections. The geometric mean age for C (3.77 years; 95% C.I. 2.77 - 5.13 years) was significantly smaller than for SC (8.7 years; 95% C.I. 6.68 - 11.3 years) (t-test on log transformed data: $t = 3.3$; 51 D.F.; $P = 0.0021$). There was no significant difference between the sex ratio of conversions in SC, 43.7% male (7/16), compared to the sex ratio in C, 47.2% male (17/36) ($\chi^2 = 0.05$, $P > 0.05$). The absence of any gender effect was confirmed by a comparison of the Force of Infection fitted by the infection-recovery model to the age prevalence data: λ in males was 0.15 (95% C.I. 0.11 - 0.18) cases/person-years (Figure 4.10a); λ in females was 0.13 (95% C.I. 0.12 - 0.14) cases/person-years (Figure 4.10b). We are unable to make any direct test of the effect of susceptibility on the risk of clinical symptoms with infection. However, as described above, there was no difference in the induration size of the MST response in people following clinical or subclinical infection.

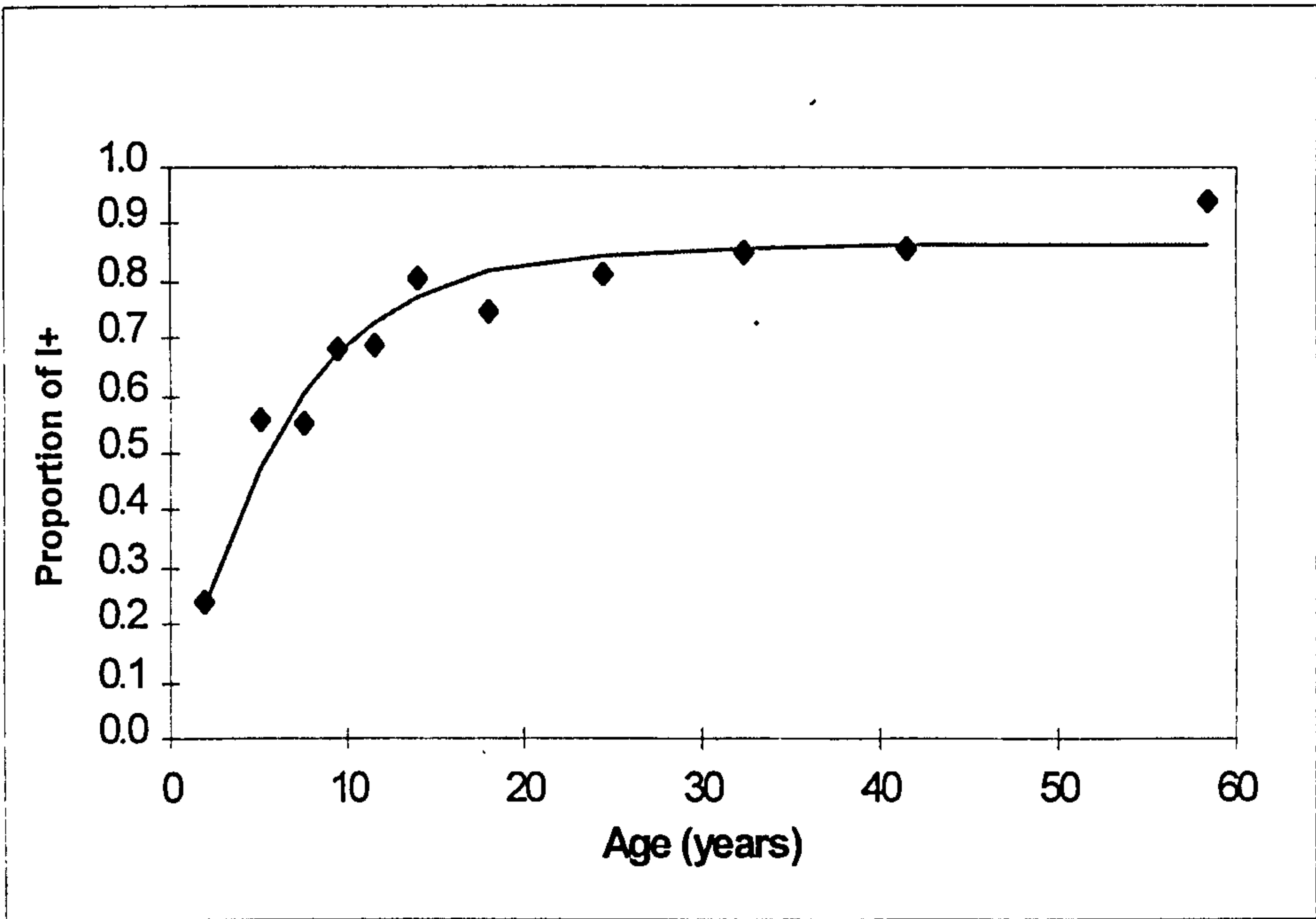
A multivariate analysis having clinical/subclinical infections as a binary response variable, and age, gender and final MST size as potential explanatory factor, resulted in a minimal adequate model containing only age ($r^2 = 0.14$). The model predicts a significant increase in the subclinical infection rate with age; for example, α is predicted to decrease from 89% at age 0 to 14% at age 30 (Figure 4.11).

Figure 4.10 Age prevalence curves of clinical infection by gender

a) Age prevalence curves of infection for males

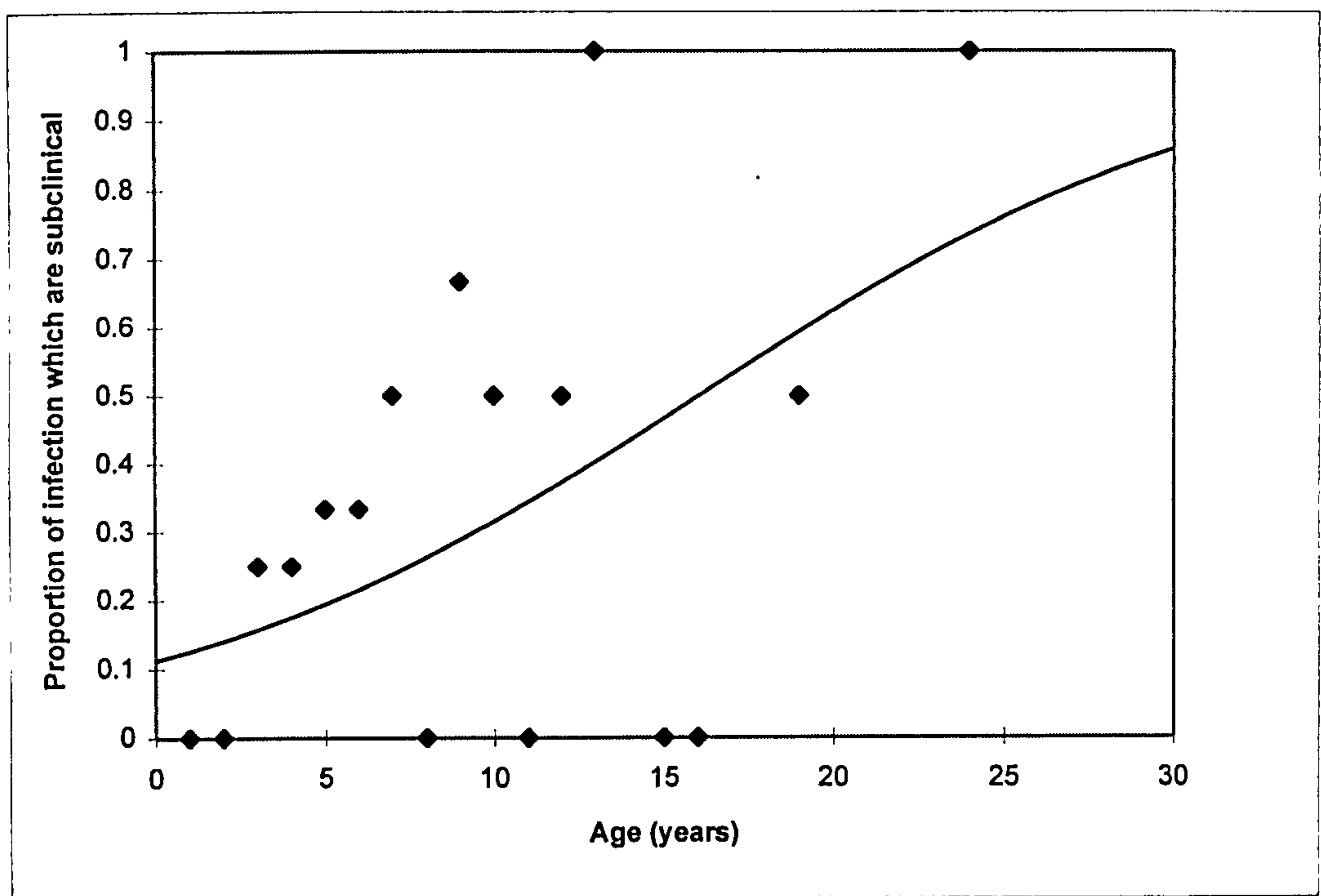


b) Age prevalence curves of infection for females



Age prevalence curves for I+ detected during the cross-sectional study. Each point represents the proportion of I+ within a particular age group. Lines were drawn by maximum likelihood fit to the infection-recovery model (Williams, 1994, see Table 4.5)

Figure 4.11 The relationship between age and the proportion of infection which are subclinical



Each point represents the observed proportion of subclinical infections. The line is the expected proportion predicted by multiple logistic regression: $\log(\text{odds}) = (-2.066) + (0.156 \cdot \text{age})$ (s.e. intercept: 0.6191, s.e. slope: 0.06314)

4.2.8 Acquired immunity against disease

During the follow up, 82 patients were detected but only 61 were enrolled, as 21 persons refused diagnostic procedures and no clinical histories were taken (Table 4.4). In order to determine the role of acquired immunity for protecting against new episodes of clinical leishmaniasis, a comparison was made between patients ($n = 61$) and non-patients ($n = 1272$).

The explanatory variables tested first in univariate analysis were village, age, gender, MST induration size in 1995 and previous lesions.

The geometric mean age in patients (5.5 years; 95% C.I. 5.25 - 5.74 years) was significantly lower than in the non-patients group (14.3 years; 95% C.I. 14.75 - 14.84 years) (t-test on log-transformed data: $t = 8.83$; 1,332 D.F.; $P < 0.0001$), indicating that the rate of clinical infection decreases with age. The geometric mean MST induration size in patients (1.43 mm; 95% C.I. 1.08 - 1.77 mm) was also significantly lower than in the non-patients group (7.6 mm; 95% C.I. 6.63 - 6.76 mm; t-test on log-transformed data: $t = 7.55$, 1,332 D.F.; $P < 0.0001$), indicating that MST size is associated with protection against disease. Irrespective of MST response, there was a significantly higher proportion of new patients in the group of healthy people₁₉₉₅ (42/410, 0.10) than in the scarred group₁₉₉₅ (19/882, 0.02) ($\chi^2 = 40.7$, $P < 0.001$, RR = 5.0, 95% C.I. 2.9 - 8.3), indicating that previous clinical disease is associated with protection against new lesions. In addition, when the analysis focused exclusively on the healthy group, there was a significant higher proportion of patients in M-L- (39/293, 0.13) than in M+L- (3/117, 0.025) ($\chi^2 = 9.37$, $P = 0.002$, OR = 5.83, 95% C.I. 1.79 - 30.03), indicating that subclinical infected people are protected against the risk of subsequent clinical infection. However, focusing on the scarred group, there was no significant difference in the clinical infection rate between M-L+ (2/30, 0.06) and M+L+ (16/852, 0.02)

($\chi^2 = 1.36$, $P = 0.12$). On the basis of univariate analyses, patients and non-patients did not differ significantly either according to gender or according to village.

A multivariate analysis was then carried out, taking incident clinical infections as the binary response variable, and village, age, gender, MST size and previous lesions as explanatory variables in the maximal model. Village and gender dropped out from the full model, as expected, producing a minimal adequate model with age, MST size, presence of previous lesions, and also an interaction effect between MST size and clinical status (Table 4.10).

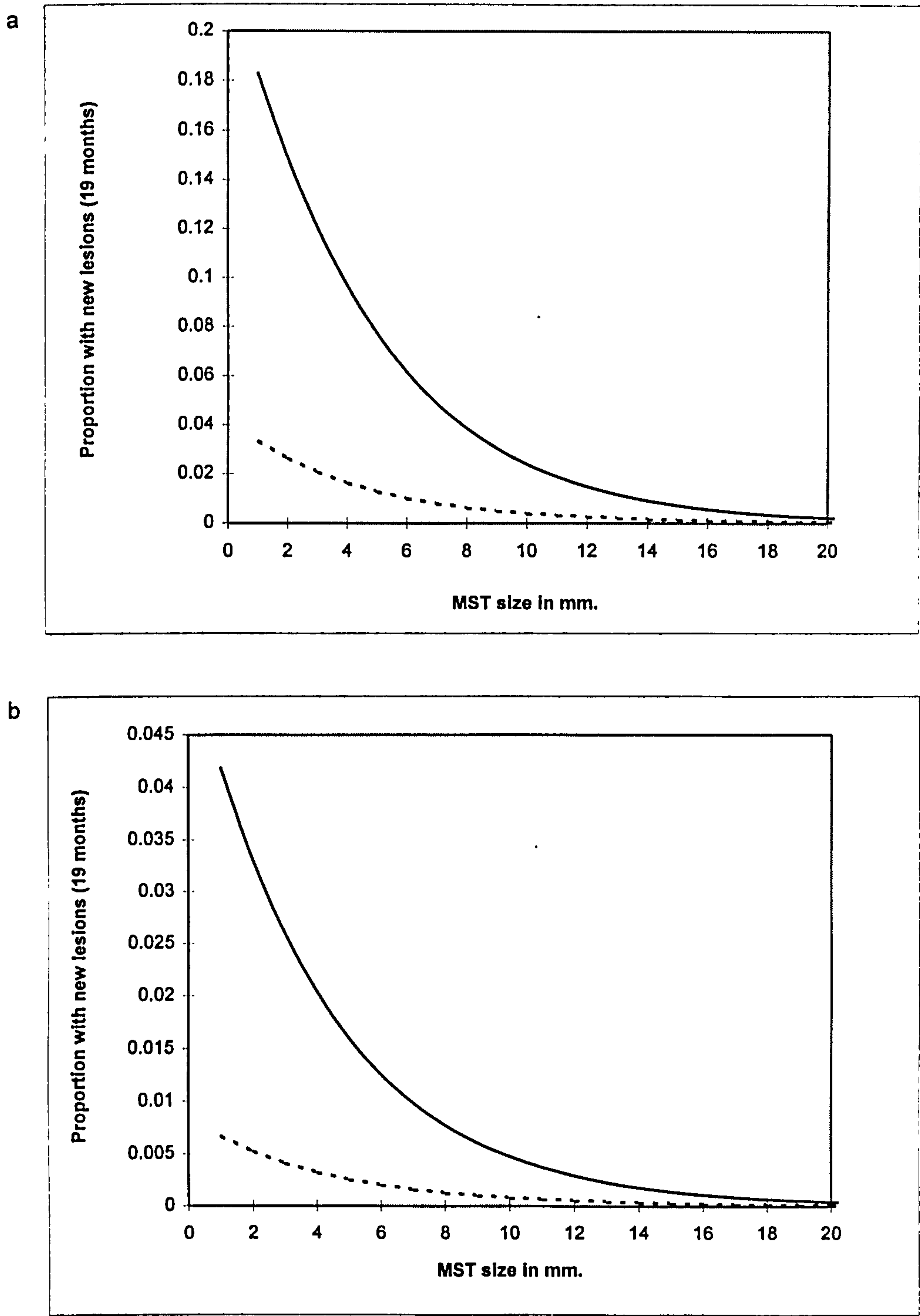
The main conclusions are as follows. (1) Irrespective of MST and clinical status, the odds of a new lesion decreases significantly with age by 5.4% per year ($1-(e^{-0.056})*100$). (2) Amongst both the healthy and scarred population, the risk of a new lesion decreases with MST size. This effect is significantly greater amongst the healthy population, for which the odds of a new lesion decreases by 22% ($1-(e^{-0.245})*100$) per 1 mm increase in MST size. This compares to an equivalent reduction of 9% ($1-(e^{-0.101})*100$) for the scarred population. (3) Irrespective of age and MST status, the odds of a new lesion is significantly less for the scarred than for the healthy population; but this effect is less marked for people with a greater MST size. For example, for a person with no MST response (0 mm), the odds of a new

lesion decrease by 87.7% ($1-(e^{-2.02}) \times 100$), whereas for people with an MST response of 10 mm the odds decrease by only 43.4% ($1-(e^{-0.57}) \times 100$). The effects of MST size and clinical status on the risk of a new lesion are illustrated in Figure 4.12a, which focuses on children aged 1 year, and Figure 4.12b, which focuses on adults aged 30 years.

Table 4.10 Minimal adequate model for risk factors of clinical infection, recovery rate and MCL

outcome	parameter	estimate	s.e	X ²	P	r ²
CLINICAL INFECTION	intercept	-1.439	0.2104			
	age	-0.05635	0.01841	14.5	< 0.001	
	MST	-0.2456	0.1257	4.18	< 0.05	0.136
	previous les.	-2.019	0.546	10.71	< 0.01	
	interaction	0.1444	0.06886	4.2	< 0.05	
RECOVERY RATE	intercept	0.9779	1.012			
	previous les.	-1.365	0.5315	5.95	< 0.05	0.16
	MST	-0.2396	0.07557	14.54	< 0.001	
MUCOSAL DISEASE	intercept	-3.193	0.3157			
	males	0.9892	0.2395	18.36	< 0.001	0.44
	MST	0.03943	0.01649	5.58	< 0.05	

Figure 4.12 The relationship between incidence of new lesions and MST size, clinical status and age



Solid lines represents "healthy" people 1995, the dotted line represents people with a previous lesion (i.e prior 1995). Figure (a) corresponds to one year old children and Figure (b) to 30 years old adults.

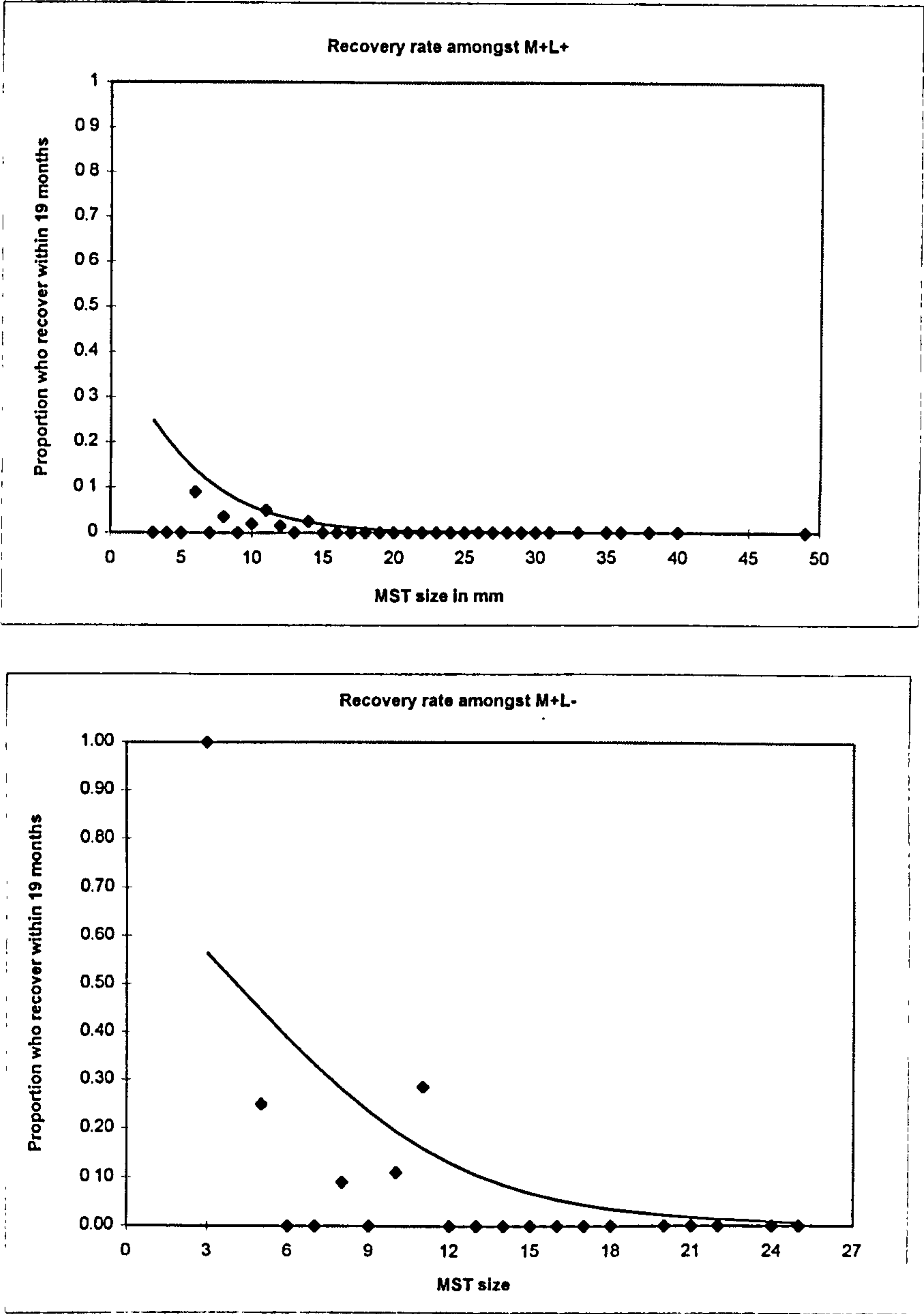
4.2.9 Recovery rate

The loss of positive MST responsiveness was detected in 16 persons from both the L+(scarred)M+ and L-M+ groups (n = 490) at a rate, ρ , of 0.024 (95% C.I. 0.018 - 0.029) cases/person-years. In the univariate analyses, the geometric mean MST induration size (at the first survey) amongst those who later recover (8.17 mm; 95% C.I. 7.99 - 8.34 mm) was significantly smaller than amongst those who remained M+ (13.13 mm; 95% C.I. 12.10 - 13.15 mm, t test on log-transformed data; $t = 4.312$; $P < 0.0001$). The rate of loss of MST responsiveness was significantly greater in people without previous lesions (7/51; 0.13; 95% C.I. 0.08 - 0.17) than amongst L+ people (9/439; 0.021; 95% C.I. 0.014 - 0.027; $\chi^2 = 16.2$; $P < 0.0001$). The mean age of those who recovered (13.6 years ; 95% C.I. 13.1 - 14.0 years) was not significantly different from those who remained M+ (16.6 years; 95% C.I. 15.58 - 17.62 years; t test on log-transformed data; $t = 1.54$; $P = 0.3$). The proportion of males amongst those who recover (7/16; 0.43; 95% C.I. 0.18 - 0.67) was not significantly different from those who remained M+ (244/490; 0.5; 95% C.I. 0.46 - 0.53; $\chi^2 = 0.05$; $P = 0.82$).

These results were confirmed by multivariate analysis, in which MST induration size and previous lesions were retained in the minimal adequate model ($\chi^2 = 25.0$; 2 D.F.; $P < 0.001$; $r^2 = 0.15$) (Table 4.10). Figure 4.13 illustrates the negative relationship between the recovery rate and

MST induration size for clinical (Figure 4.13a) and subclinical (Figure 4.13b) infected people. It shows that M+L- people are more likely to recover to M- than are M+L+ people.

Figure 4.13 The relationship between the loss of MST responsiveness and both MST size and clinical status

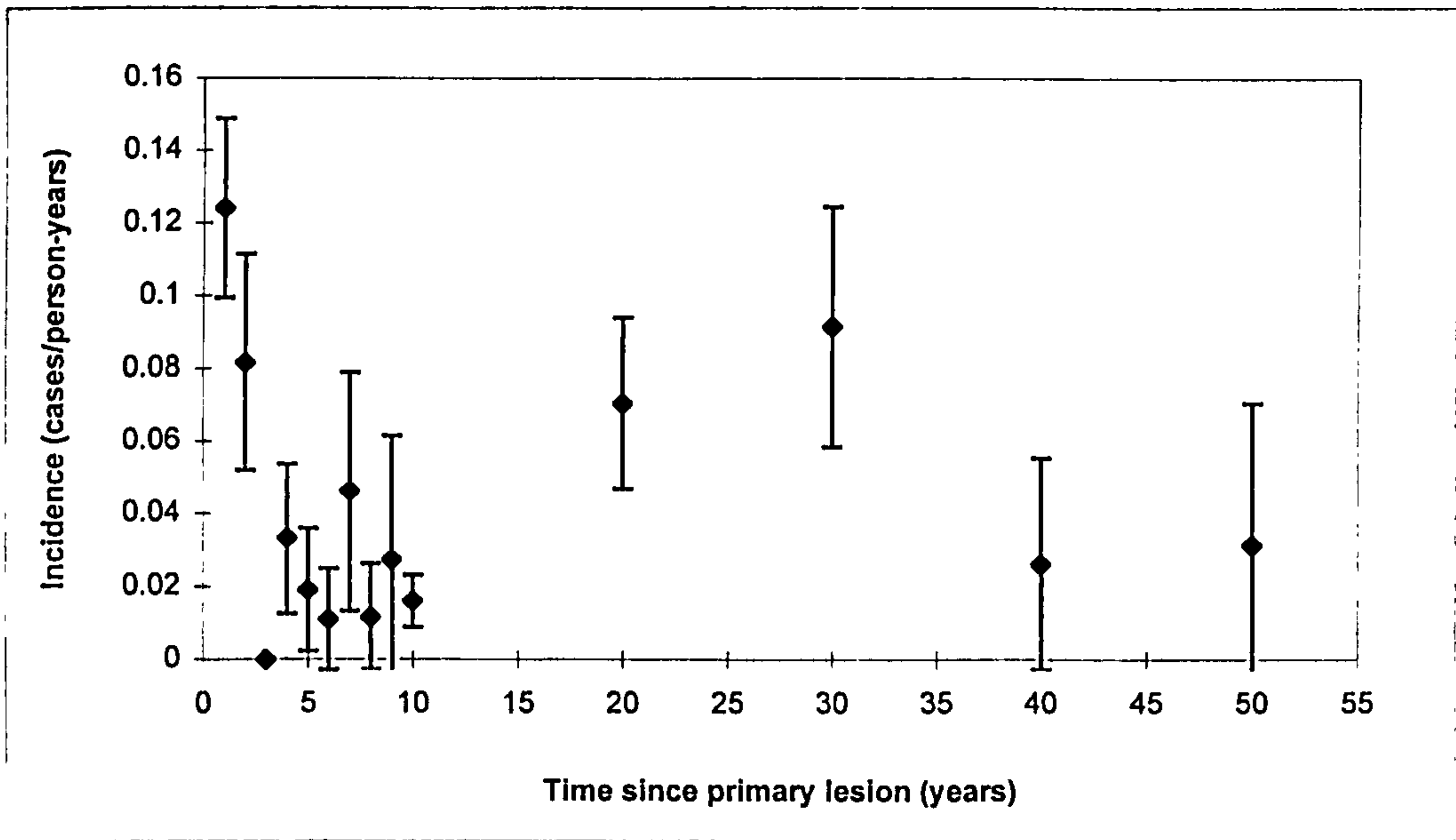


Diamonds represent the observed recovery rate. The line was fitted by multiple logistic regression (see Table 10)

4.2.10 Recurrent leishmaniasis

Previous leishmaniasis scars were detected in 80/119 (67.2%) patients enrolled during the study. Figure 4.14 illustrates the mean incidence rate of secondary lesions with respect to time since primary lesions in the scarred population. Incidence decreased from 0.12 to 0.016 cases/person-years in the first 10 years after the primary lesion, indicating that these early secondary infections were relapses rather than re-infections. However, 15 years after the primary infection, incidence rates increase to a peak of 0.091 cases/person-years, which is not significantly different from the incidence in L-1995 (0.1 cases/person-years) (Figure 4.14).

Figure 4.14 The annual incidence rates of secondary lesions according to time since the primary lesion



The X axis refers to the first year of the time periods during which each mean incidence rate was calculated. Incidence rates are presented on the Y axis with 95% confidence intervals

The precise localisation of lesions and the time since primary lesions occurred were recorded on the clinical history forms. For 34% (21/61) of the patients, the secondary lesions appeared in the same site in the body as the previous scars. For this population, the mean time between the primary and secondary lesions was 4.89 years (median 2 years). In contrast, the mean time between episodes when secondary lesions were on a distant site was 9.25 years (median 3 years) which was significantly higher than for the former (H (Kruskal-Wallis) = 4.83, P = 0.027, 1 D.F.)

4.2.11 Seasonal transmission

There is some evidence of seasonal variation in the incidence of clinical leishmaniasis (Figure 4.15 and Figure 4.16). From November 1994 (considering the evolution time of chronic lesions detected during the cross-sectional study) to January 1997, the peak number of primary leishmaniasis cases (parasitologically diagnosed) (Figure 4.15a) were 8 in December 1995, 7 in March 1996, and 4 in each of December 1994, February 1995 and February 1996 (suggesting a peak between December-March). Zero cases were detected in July and August 1995, and again in June and July 1996, suggesting a trough between June - August which, according to the rainfall patterns, is the second driest period in the year (see Materials and Methods). In contrast, no seasonal pattern was observed for the incidence of recurrent

cases (Figure 4.16a,b), which is consistent with the hypothesis that most secondary infections are re-activations rather than re-infections. Similarly, suspected but unconfirmed primary cases were distributed randomly throughout the year, indicating some misdiagnosis (Figure 4.15b).

Figure 4.15 Monthly incidence of primary leishmaniasis cases

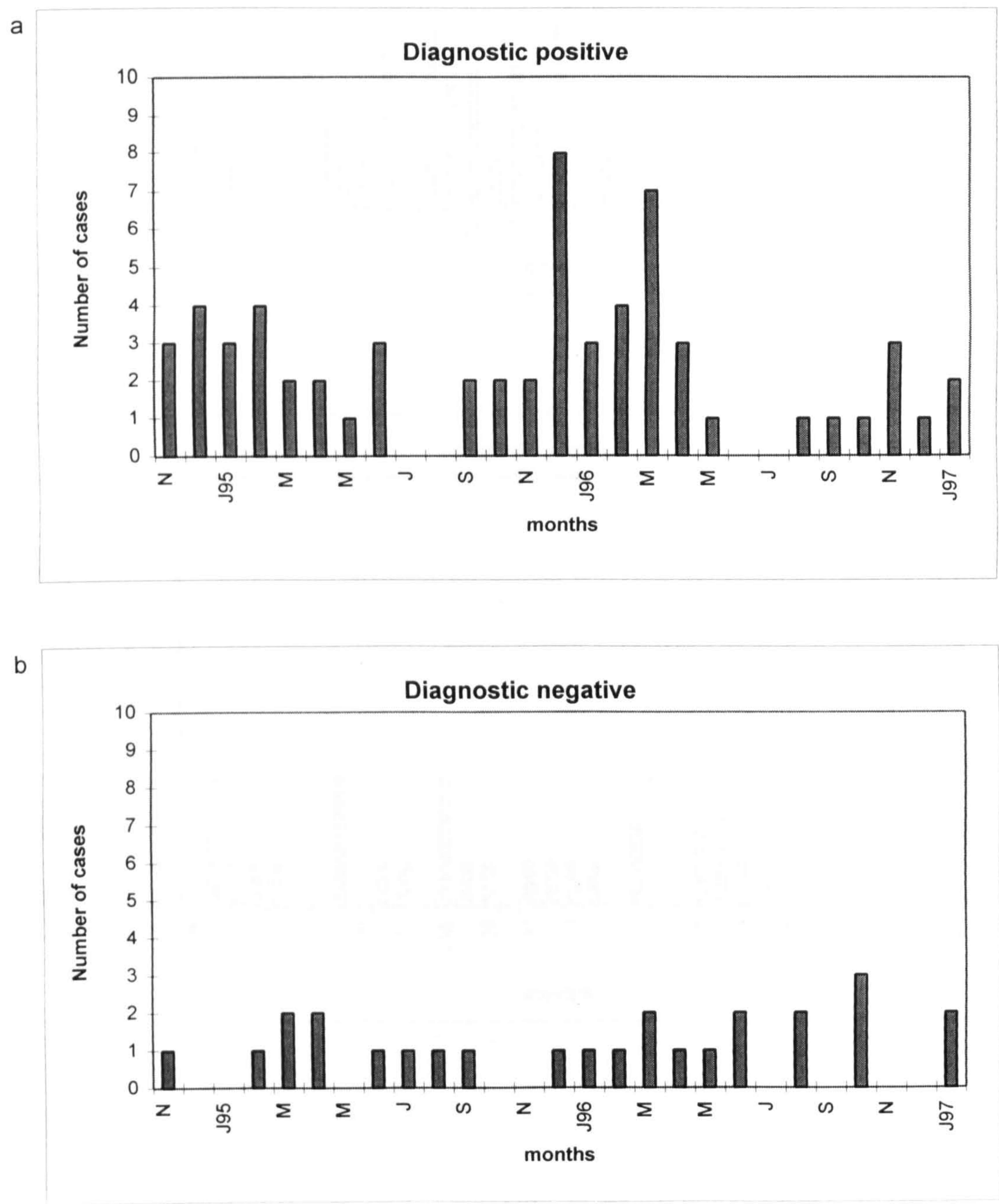
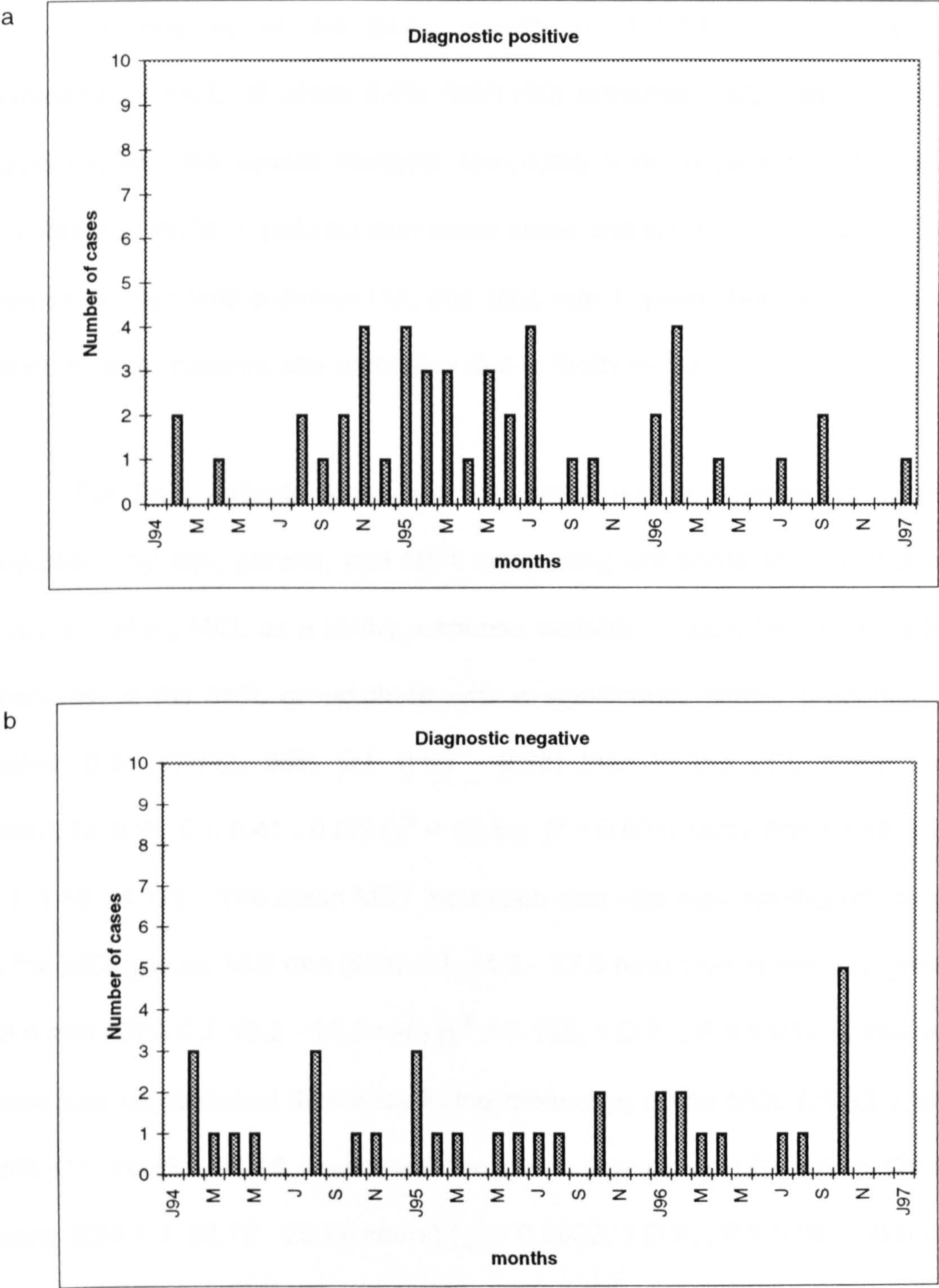


Figure 4.16 Monthly incidence of recurrent leishmaniasis cases



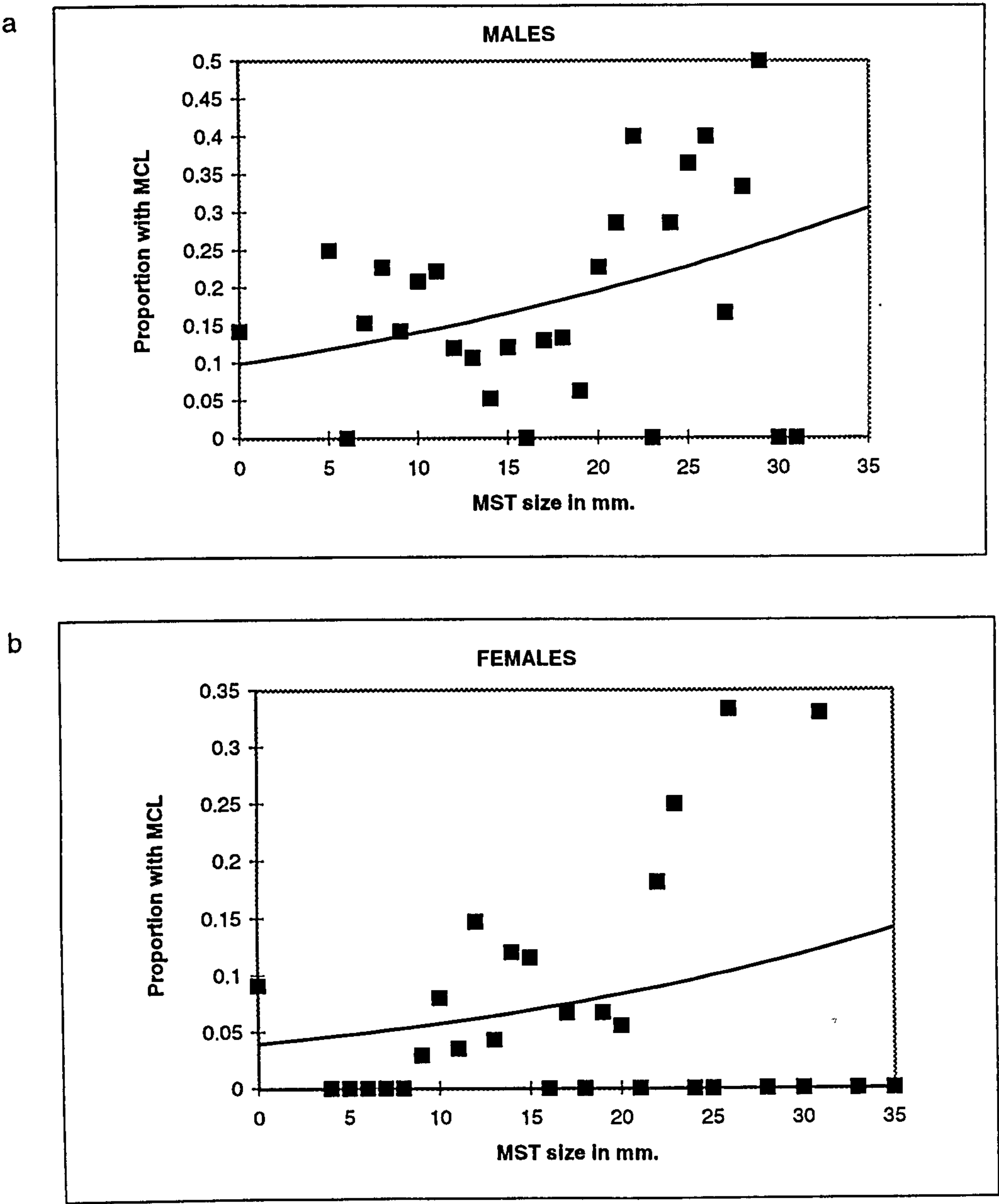
4.2.12 Mucocutaneous leishmaniasis (MCL)

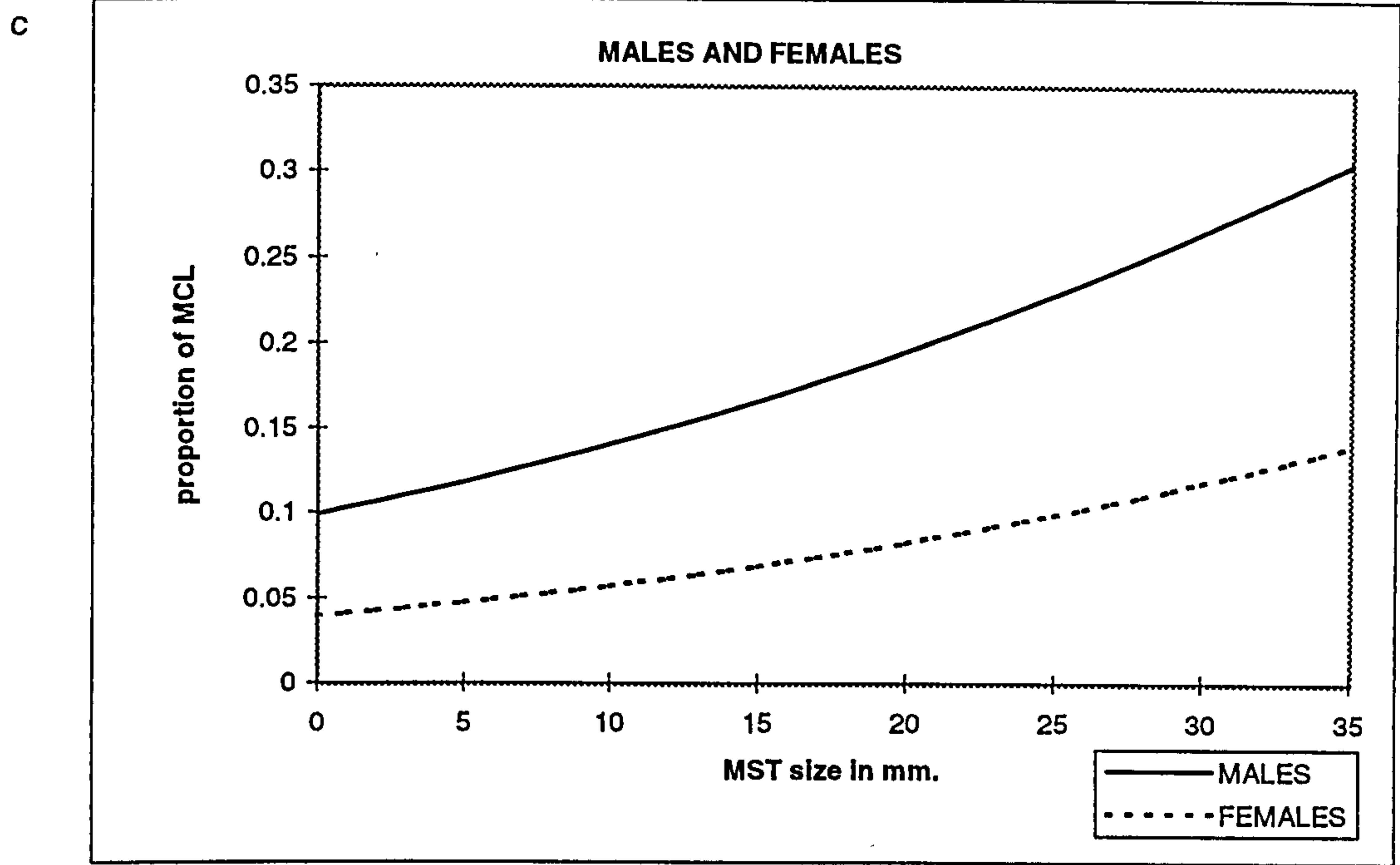
The majority of the study population (1100/1380) was clinically inspected for MCL, of which 8.4% (92/1100) presented large lesions in the nasal septum. No severe mucosal symptoms were detected in the study population. All MCL patients presented scars and were MST positive. The mean evolution time between LCL and MCL was 9 years, but the information collected from patients was unreliable due to faulty recall.

The MCL patients group was compared with the remaining scarred population by age, gender, and MST size, using univariate and multivariate analysis, taking MCL as a binary response variable. According to univariate analyses, in the MCL group there was a significantly higher proportion of males: 0.69 (64/92, 95% C.I. 0.59 - 0.78) than in the LCL group 0.47 (384/802, 95% C.I. 0.41 - 0.52) ($\chi^2 = 15.51$; $P < 0.001$; Odds Ratio 2.49, 95% C.I. 1.53 - 4.12). The mean MST induration size was also significantly larger in the MCL group: 15.6 mm (95% C.I. 14.2 - 17.0 mm) than in the LCL group: 13.6 mm (95% C.I. 13.2 - 14.1 mm) ($\chi^2 = 7.168$, 1 D.F., $P < 0.01$). However, there was no statistical difference in the mean age of the MCL (23.32 years, 95% C.I. 19.48 - 27.15 years) and the mean age of the LCL group (25.04 years, 95% C.I. 23.79 - 26.28 years) ($\chi^2 = 0.8032$, 1 D.F., $P > 0.05$). Gender and MST size were retained in the minimal adequate model fitted by multivariate analysis (Table 4.10), the results of which are illustrated in Figure

4.17: The model predicts a positive relationship between MST size and the proportion with MCL for both genders, and a higher MCL rate in males than in females (irrespective of MST size). The strength of the association was relatively high: $r^2 = 0.44$ (Table 4.10), i.e. 44% of the variance in the MCL rate was explained by gender variation and by MST size.

Figure 4.17 Personal risk factors for MCL





In Figure a and b squares represents the observed prevalence of MCL in males and females. The solid line represents the predicted prevalence of MCL. Figure c contains the same values of predicted prevalence in both gender without observed values

4.2.13 Identification of likely sources of bias

4.2.13.1 Potential bias through the selection of the “study population”

The majority of the “excluded population” belonged to a Christian fundamentalist group who typically refuse to participate in health programs although they do accept medical treatment when required. The census was applied to this population during the cross sectional study but the clinical exploration was restricted only to legs, arms and head of males and latter two in females. As a result of this lack of information, there was a significantly small proportion of persons with scar or lesions in the “excluded population”

than in the “study population” (Table 4.11). 22 patients from the “excluded population” were registered during the follow up by passive search, producing an incidence rate of 0.01 cases/person-years at risk, which was (as expected) significantly lower than the incidence in the study population ($\chi^2 = 33.46$, $P < 0.0001$).

The low leishmaniasis prevalence and incidence in the “excluded group” reflect only the variation in the acceptance of this population to the leishmaniasis project, rather than different level of exposure. Both groups had similar type of houses which were located randomly in the 12 villages under study, and both groups did the same type of work (i.e. harvesting of tropical forest, cacao plantations, etc.). During the follow up survey, the active search of cases in the “study population” of each village received permanent help from the local trained person (see Material and Methods), whereas the passive search “survey” only operated during the periodical visits of the medical team.

Apart from the group of religious people, some heads of families of the “study population” accepted the importance of the leishmaniasis project only for females and children but not for males. This generated a distinct population structure (age and gender) in the “excluded population”, i.e. the excluded group was significantly older and more male biased (Table 4.12). This bias probably had little effect in the measurement of incidence, as all

excluded persons “heads of families” presented scars at the time of the first cross-sectional study and no reactivations were detected during the follow up.

4.2.13.2 Potential bias through incomplete follow-up of the study population

Two sub-groups were lost during the follow-up: emigrants and people who refused the second MST (Figure 4.2). The bias introduced by emigration could affect the measurement of both clinical and subclinical infection rates, whilst those who refused to accept a second MST could only affect the measurement of subclinical rates because the “refusal” group was followed-up by clinical inspection throughout the survey.

a) Emigrants

There was no significant difference by gender between emigrants (E) and the population with second MST (SM), but emigrants were significantly older than the remaining population (Table 4.13). There was no significant difference between the proportion of scars in E, 0.68 (119/173; 95% C.I. 0.61 - 0.74) and SM, 0.63 (879/1380; 95% C.I. 0.60 - 0.65; $\chi^2 = 1.59$, $P = 0.20$). Also, the proportion of lesions were not significantly different between E, 0.05 (10/173, 95% C.I. 0.017 - 0.082) and SM, 0.065 (90/1380; 95% C.I. 0.051 - 0.078; $\chi^2 = 0.01$, $P = 0.91$). Emigration is an unlikely source of bias because

it is motivated by economic reasons, rather than health problems (i.e., leishmaniasis).

b) Refusal group

At the end of the follow up, 36.5% (441/1207) of the study population (still in the area) refused the application of the second MST. This group was significantly older than SM but there was no difference by gender (Table 4.14). The great majority of refusals were adults with a positive result in the first MST. Thus, the main possibility of bias due to the refusal would apply to the measurement of recovery rate, ρ , rather than the measurement of transmission rate.

Table 4.11 Comparison of the study and excluded populations by clinical status

CLINICAL STATUS	STUDY POP.	EXCLUDED POPUL.	odds ratio	95% C.I.	χ^2	P
HEALTHY	411	482				
SCARS	879	800	1.29	1.09-1.52	9.33	<0.0002
LESIONS	90	42	2.51	1.68-3.80	22.57	<0.0001
TOTAL	1380	1324				

Table 4.12. Comparison of the study and excluded populations in the first MST application by age and gender

		STUDY POPULATION	EXCLUDED
AGE	OBSERVATIONS	1333*	1106*
	MEAN	19.974	22.82
	VARIANCE	273.87	320.7
	SIGNIFICANCE TEST	Kruskal-Wallis H (equivalent to χ^2) : 17.767	
	P	< 0.0001	
GENDER	FEMALES	688	576
	MALES	692	748
	SIGNIFICANCE TEST	χ^2 (Yates corrected) = 10.69	
	P	= 0.001	

Table 4.13. Comparison of the study population and emigrants by age and gender

		STUDY POPULATION	EMIGRANTS
AGE	OBSERVATIONS	759	155
	MEAN	18.2	20.8
	VARIANCE	259.55	263.94
	SIGNIFICANCE TEST	Kruskal-Wallis H (equivalent to χ^2) : 7.303	
	P	0.006	
GENDER	FEMALES	386	87
	MALES	381	81
	SIGNIFICANCE TEST	χ^2 (Yates corrected) = 0.07	
	P	= 0.79	

Table 4.14. Comparison of the study and the refusal group at the second MST survey

		STUDY POPULATION	REFUSAL
AGE	OBSERVATIONS	759	415
	MEAN	18.2	22.89
	VARIANCE	259.55	290.49
	SIGNIFICANCE TEST	Kruskal-Wallis H (equivalent to χ^2) : 31.799	
	P	< 0.0001	
GENDER	FEMALES	386	213
	MALES	381	228
	SIGNIFICANCE TEST	χ^2 (Yates corrected) = 0.38	
	P	0.53	

4.3 DISCUSSION

The main rationale for epidemiological studies of leishmaniasis is to improve the effectiveness of treatment and to enable the establishment of control strategies. An overview of the natural history of LCL caused by each parasite in different foci in Central and South America will only be possible once a series of long-term follow up studies (FW) is carried out in different countries (i.e. in foci with different ecological characteristics). However, these studies often require following a highly dispersed population in remote areas several times per year. Such an approach is expensive, time consuming, and, in some Latin American countries in which there is paramilitary and guerrilla activities, dangerous. To date, the large majority of studies reported in the literature are cross-sectional studies (CX), conducted in areas where some action was required after an outbreak of leishmaniasis had been notified. A number of studies have also focused on new settlements where transmission to humans was a new phenomenon, using as denominators only the number of people at high risk of transmission during the epidemic. Table 4.15 summarises the most relevant studies using this approach, and many of these studies will be referred throughout this section.

Only three long-term follow up studies of LCL have been conducted (and reported) in South America: in Peru, Colombia and Brazil. (1) The Peruvian study was conducted by Davies et al (1995a) from 1991 - 1993 in 38

villages located in the naturally unforested Andes mountains (Departments of Lima, Ancash and Piura), between 1,500 and 3,000 m above sea-level (m a.s.l.). The study population comprised 4,716 people, data concerning demographic and clinical aspects were collected, and a MST was carried out, on 72% of the population at the first survey; 480/877 (55%) of the MST negative people were re-tested in the second survey. (2) The Colombian study was carried out from September 1986 to December 1989; by Weigle et al (1993) in a study population of 2,858 persons living in 15 contiguous villages (in an area of 27 km²) in the municipality of Tumaco, Narino (Pacific lowland coast). This region is 10 - 30 m a.s.l. and covered with tropical rain forest (now partially deforested). In this study, as in the Peruvian study, MST was applied sequentially. (3) The Brazilian study was conducted by Jones et al (1987) from 1980 to 1984 in 15 farms and villages in Tres Bracos, Bahia. This region is located between 600 - 800 m a.s.l. and covered with remanent patches of tropical rain forest, with large cleared areas planted with cacao, banana and manioc. This study was carried out using only clinical and demographic data, MST was only used as a diagnostic tool in suspected patients. In the Colombian study, the most common parasite was *L. panamensis*, which was also the only parasite isolated in Opon (see Chapter 3). In Brazil, the parasite was *L. braziliensis* and in Peru *L. peruviana*.

Table 4.15 Previous relevant studies of leishmaniasis epidemiology in Central and South America

COUNTRY	REGION	STUDY AREA	H@	T@	OUTCOME		POPUL. SIZE	CLINICAL PREV.	MST PREV.	CLIN. INCID.	SUBCLIN. INFECT.	RISK FACTORS		AUTHOR	YEAR
					CX	FW						AGE	GENDER		
BRAZIL	Sabara, Belo Horizonte	2	2	2	CLIN +MST	ND	1,119	0%	31/881; 3.5%	ND	7/31, 23%	6 - 16	females	Passos	1993
	Nova Iguaçu, Rio	3	2	2	CLIN +MST	CLIN	137	18/137; 13%	24/137; 17.5%	NS	6/119, 5%	< 15	males	Oliveira-Neto	1988
	Sao Paulo	3	1	1	CLIN +MST	CLIN	355	22/273; 8%	22/86; 25.5%	max. 71.5/10,000	16.30%	5 - 29	females	Gomes	1992
	Jacarepagua, Rio	2	4	2	CLIN+MST	ND	731	20/731; 2.7%	46/731; 6.2%	ND	26/46; 57%	NA	equal	Souza	1992
	Rio Doce Valley, Minas Gerais	3	1	2	CLIN+MST	ND	404	181/404; 45%	163/404; 40.3%	ND	4/404; 1.8%	5 - 30	equal	Barreto	1981
	Itaporanga, Sao Paulo	3	1	NS	CLIN+MST	ND	231	active: 67/231; 29%	66%	NS	NS	2 - 80	equal	Stolf	1993
	Corte de Pedra, Bahia	3	1	NS	CLIN+MST	ND	1,048	active 25,4/1,000	NS	max. 88.3/1000	NS	0 - 9	equal	Franca	1991
	El Aguacate	3	1	3	CLIN	ND	411	140/411; 34%	ND	ND	ND	NS	NS	Herrer	1976
PANAMA															
COLOMBIA	Dagua	3	1	1	CLIN	ND	1,127	27/1,127; 2.3%	ND	ND	ND	< 15	equal	Montoya	1990
PARAGUAY	Limoy	4	5	1	CLIN+MST	ND	149	88/149; 59%	74/149; 50%	ND	8/149; 5%	NS	equal	Hashiguchi	1991
COSTA RICA		3	1	NS	CLIN+MST	ND	348	166/348; 48%	187/348; 54%	ND	21/348; 6%	< 15	equal	Marrano	1989
VENEZUELA	Merida	1	3	NS	CLIN+MST	ND	2,530	545/2,530; 22%	490/2,530; 19.3%	ND	14/2,530; 0.8	18 - 20	equal	Scorza	1983

h@ = HABITAT TYPE

habitat 1: tropical rain forest with large clearness of cacao, banana and manioc

habitat 2: periurban areas/deforested

habitat 3: range of vegetation from forest to xerophitic

habitat 4: secondary forest

habitat 5: tropical rain forest, recent intervention

T@ = PLACE OF TRANSMISSION

trans 1: around or in houses at the age of the forest or crops like coffee or cacao

trans 2: inside houses-periurban areas

trans3: disease in individuals in forested areas during work or play activity

Outcome: CX = cross-sectional studies; FW = follow up studies. CLIN = outcome clinical symptoms; MST = MST response

ND = not done; NS = not specified

* How the area was chosen

Choose the area: 1: cases provenance and then random selection of villages

area2: low and high risk villages

area 3: area with high transmission, not specific selection

area4: new settlement

Davies et al, 1995; Weigle et al, 1993; Jones et al, 1987; are described in the main document

In the Opon focus, the study of risk factors for infection are intended to be the starting point for future control programs ; and the study of risk factors for disease should contribute to future programs aiming to detect and supply treatment to all cases in groups at high risk of severe forms of leishmaniasis. The risk factors for infection included gender, age, previous clinical status, localisation of the household, village and season. The risk factors for disease included age, gender, and immune status. The outcome parameters include the risk of developing mucocutaneous leishmaniasis, the risk of reactivation, subclinical infections, and efficacy of treatment.

4.3.1 Transmission rates

The Opon region is one of the most highly endemic foci of LCL in the Santander Department, Colombia. The cumulative prevalence of clinical leishmaniasis (I+) was 0.72 cases/person, which was not significantly different from the cumulative prevalence of infection amongst the whole study population (m+), 0.75 cases/person (Table 4.4). This prevalence should be representative of the Landazury municipality, since the villages under study were not selected on the basis of their suspected prevalence, but were chosen to reflect the different habitats created by deforestation. These cover the whole range of activities carried out by Landazury farmers: from cattle farming to cacao cultivation, and including land use for temporary crops like

maize and yuca (manioc), plus secondary forest. The transmission rate in Landazury was similar to that recorded in some valleys in Peru ($I^+ = 0.737$; $m^+ = 0.78$), but higher than in both Tres Bracos, Brazil ($I^+ = 0.15$) and in Tumaco, Colombia ($m^+ = 0.093$). In the previously reported CX study of LCL, the reported cumulative prevalence (m^+) ranged from 0.023 to 0.59/person (Table 4.15). In general, the smaller studies recorded higher prevalence as they focused on small (unrepresentative) populations who were expected to have a high risk transmission rate.

The incidence of infection in the M-L- group in the Opon focus, 0.19 conversions/person/years, was similar to the incidence of infection recorded in Peru, 0.11 conversions/person/years, but almost three times higher than in Tumaco, where the incidence of infection was 0.066 conversions/person/years. In Tumaco, the pattern of exploiting tropical wood is different from that in the Opon focus, where deforestation has been gradual with the preservation of some trees from the primary forest. In Tumaco, in contrast, deforestation tends to be quick and total, followed by the cultivation of temporary crops like plantain and isolated fields of cacao crops far away from houses. Thus, one explanation for different incidence rates between Tumaco and Opon is related to the level of adaptation of vectors and reservoirs to the environmental changes characteristic of the two foci.

4.3.2 Risk factors for infection

4.3.2.1 Personal risk factors

Personal risk factors for infection are most accurately estimated from FW studies with two assessments of MST status in the population. Correlates with infection rate can then be identified either by cohort studies (as in the Opon, Tumaco and Peruvian studies) or by case-control studies (e.g. Llanos-Cuentas, 1993). In contrast, retrospective studies of infection, whether by case-control studies (e.g. Yadon, 1996) or cohort studies, are plagued by potential bias and other inaccuracies associated with faulty recall or changes in circumstance. Thus, the Opon focus results are here compared with the Tumaco and Peruvian results. New infections occurred irrespective of age and gender in Opon and in Peru, whereas in Tumaco incidence of infection increased with age, and infection was three times greater in males than in females. These differences are thought to be mainly the result of differences in exposure rather than differences in susceptibility (see p. 143-144). The average age of infection in Opon, 7.7 years ($1 / \lambda$), calculated from the MST data (CX), and the absence of gender or age as a risk factor (FW) is highly indicative of intradomiciliary or peridomiciliary transmission, as is the case in the Peruvian Andes. Whereas, in Tumaco, infection is mainly an occupational hazard (farming), and this occupation in Colombia is carried out principally by males. The contrast with Tumaco is

striking, given the similarities between Opon and Tumaco both in the agent of disease (*L. panamensis*) and in the presumed principal vectors (*Lu. trapidoi*, and *Lu. gomezi*), see Chapter 5.

In previously reported case-control studies in Central and South America, households with characteristics similar to the Opon houses have been identified as risk factors for leishmaniasis: for example, houses on stilts (OR= 3.2; 95% C.I. 0.64 - 18.9) (Rojas et al., 1988) and houses with hole(s) in the walls (OR= 8.0; C.I. 2.5 - 25.5) (Yadon, 1996). Thus, in future, control programs in the Opon area should perhaps be orientated towards house improvement and the encouragement of barriers (e.g. bed-nets) between sandflies and humans, in order to avoid infections in children, who are the high risk group for severe lesions, especially in the head (see Chapter 3).

4.3.2.2 Risk factors associated with land use

Much of the inter-village variation in prevalence or incidence of infection in Opon was explained by the variation in the percentage of land around houses which is covered with cacao or secondary forest. These results are consistent with the results of the study of the effect of surrounding vegetation on the indoor activity of sandflies (see Chapter 5), where cacao crops appear to provide an appropriate environment for the survival and reproduction of the principal sandfly vectors(s) in Opon.

The variance in the village transmission rates explained by the relative proportion of land covered with cacao or secondary forest indicates that land use after deforestation is one of the most important factors for leishmaniasis transmission between villages. On a larger scale, this could be one of the factors explaining the differences in transmission rates between regions (for example, the difference in transmission rates between Tumaco and Opon). In the Opon focus, the majority of households are scattered and act as centre points for farms (see Materials and Methods). A similar pattern was observed in two settlements in Tumaco (Campamento and La Quince) where most households were surrounded by tropical forest, and these two settlements presented the highest prevalence of leishmaniasis. But in general, Tumaco settlements were characteristically located one to five km distant from cultivated fields.

The effect of vegetation as a risk of leishmaniasis has been demonstrated in a number of previous studies. For example, in one of the CX studies (Alto Beni, Bolivia) houses located 100 m from the nearest forest presented a smaller relative risk for cutaneous leishmaniasis than houses located 10 m away (Alcais et al, 1997a). In previous risk factor studies, houses located within 200 m of agricultural land had a significantly high risk of LCL: for example, maize and alfalfa (Yadon, 1996). Indirect evidence for the association between vegetation types and the risk of leishmaniasis has also come from entomological studies, which have indicated a relationship

between sandfly vectors and specific crops (See Chapter 5), e.g. coffee (Scorza, 1985; Alexander et al, 1992) and cacao (Franca et al, 1991; Jones et al, 1987). Hence, future control programs in Opon should include community education programs in order to discourage people from entering high risk areas after dusk. In Opon, adults often enter agriculture areas after dusk for bathing, whilst children use these areas for play.

4.3.2.3 Seasonality

There is some evidence of seasonality in the incidence of primary cases in Opon with a peak between December to March during the second dry period of the year, which follows a rainy season which is characterised by a low frequency of rainy days unlike the first rainy season. A trough was observed between June to August during the first dry period of the year (i.e. following a rainy season characterised by abundant frequency of rainy days). In Venezuela (Feliciangelli, 1987c), as in Opon, the incidence of cutaneous leishmaniasis peaks between December to January with a trough between June - July, but the rainfall patterns are opposite to Opon, i.e. the wettest months are June, July and December. The seasonal patterns could be associated with changes in the population of the sandfly vector (see Chapter 5) or changes in human activity (e.g. fruits harvesting).

The detection of seasonal patterns in leishmaniasis transmission has a great impact on health policy programs. For example, in Landazury Municipality, Glucantime® is received from the Colombian Ministry of Health towards the middle of the year, and such supplies are promptly used (probably on recurrent lesions), leaving the majority of incident cases (especially children) at the end of the year without treatment. Thus, in Municipalities like Landazury where Glucantime® is scarce, it may be necessary to limit the use of drug for treatment for adults with recurrent lesions, in order to keep enough drugs for the incident cases in children expected between December and March. Drugs could be saved by applying short courses of intralesional treatment to adults with recurrent lesions.

4.3.3 Risk factors for clinical/subclinical infection

Clinical infection is determined by the host-parasite interaction which includes host genetic factors, acquired resistance to infections and variation in parasite pathogenicity. The host-parasite interaction can be partially assessed in cohort studies by measuring the proportion of infection which lead to disease (α), and by directly measuring acquired immunity against disease, and the recurrence rate (Davies et al, 1995a, Saravia & Weigle, 1996). The "relative pathogenicity (R)" of the parasite population can be estimated from the ratio $M+L+ / M+$ in the human population (Weigle et al,

1993), but interpretation should be made with caution (Davies et al, 1995a). The most reliable estimate of the proportion of infections causing clinical symptoms comes from follow-up studies.

In Opon, the best estimate for the proportion of subclinical infections in the Opon focus was 30.8% (FW). This is higher than the rate reported in Peru (17%) but lower than that reported in Tumaco (88.1%). This result is consistent with the hypothesis that *L. panamensis* in Opon is more pathogenic than in Tumaco, but *L. peruviana* is more pathogenic in turn than both populations of *L. panamensis* studied in Colombia. However, alternative explanations for these apparent geographic patterns are possible, as the differences may represent methodological inconsistencies between the three research groups, i.e. different sensitivities either of the clinical or MST diagnosis (due to different field workers or different leishmanin). In addition, geographical differences could be explained by different levels of cross-reacting parasitic infections in the three regions such as lizard *Leishmania* (Manson-Bahr, 1987). However, the proportion of subclinical infections may well be influenced by factors related to parasites (e.g. virulence) and/or with human susceptibility. In Tumaco the majority of the community are Negros who are infected when adults, whilst in Opon the people are whites (Figure 2.13) and "mulatos" (i.e. a mixture of Indigenous, Spaniards and Negros) infected in childhood. Evidence that people who became clinically infected in Tumaco were innately more susceptible than those who were subclinically

infected came from an experimental study, where the *in vitro* infection rate of peripheral blood monocytes with *L. panamensis* was higher in LCL patients (from Tumaco) than in asymptotically infected persons (Robledo et al, 1994). Further evidence for genetic variation in susceptibility comes from comparison of leishmaniasis symptoms in Amerindians and mixed race people in Bolivia (Alcais et al, 1997b)

In the Opon focus, the geometric mean age for clinical infections (3.8 years) was significantly smaller than that for subclinical infections (8.7 years); but there was no significant difference between the sex ratio of conversions in both groups; and there was no differences in the induration sizes of the MST response in people following clinical or subclinical infection. A positive relationship between age and the proportion of subclinical infections was also observed in Peru where the geometric mean age for clinical infection was significantly lower than those with subclinical infections (7.7 and 15.01 years, respectively); as well as in Tumaco. This pattern was also observed in a cross-sectional study in Ecuador (Armijos et al, 1997) where the age group at greater risk of clinical leishmaniasis were apparently children under five years.

The decreasing incidence of clinical infection with age could be related to undetected acquired immunity following infection (Saravia & Weigle, 1996) For example, there was a three-fold higher risk of clinical disease for migrants compared to native inhabitants in Alto Beni, Bolivia (Alcais et al, 1997a).

Alternatively, adults may be innately less susceptible to LCL (Alcais et al, 1997b) as they are to VL (Dye & Williams, 1993), even in the absence of prior exposure to leishmaniasis. Evidence for innate development of protection during childhood come from a segregation analysis of 77 migrant families in Bolivia, in which the penetrance estimators showed that younger subjects are genetically more susceptible than older subjects to LCL (Alcais et al, 1997b).

In the Opon focus, the leishmaniasis transmission patterns appear to be changing in recent years, according to the disparity between the incidence rate calculated from the CX and prospective survey analyses. The incidence rate of infection during the follow up study was greater than the force of infection calculated from age prevalence data, indicating that the infection rate during the study was greater than in previous years. However, the clinical infection rate between 1995-1997 was relatively low, perhaps due to a change in the pathogenicity of the parasite population. One other factor that could explain the disparity between the FW and CX studies is the significant difference in the recovery rate of MST responsiveness following subclinical or clinical infections. Subclinically infected people apparently revert to M- relatively quickly, so that despite a relatively high transmission rate, only a small proportion of people sampled in the cross-sectional survey retain their M+L- status.

4.3.3.1 Recurrent leishmaniasis

Recurrence of LCL is defined as the onset of active lesions in patients with a history of previous episodes of leishmaniasis. It is important because of the risk of mucosal involvement (especially with *L. braziliensis* complex parasites), and also because of the extra cost incurred in the treatment of recurrent cutaneous lesions. In the Opon focus, two important considerations were taken into account in the measures of recurrent leishmaniasis: (1) the level of recurrence amongst the treated group of patients following the WHO protocol (see Material and Methods), and (2) the characteristics of recurrences amongst patients with previous scars.

The recurrence rate is thought to be determined by (1) the species of parasites, (2) the treatment received in the first episode of leishmaniasis and (3) the immunological competence of the host (Saravia & Weigle, 1996). During the prospective study in the Opon focus, all cases detected were treated with 20 mg/kg/d of antimony for 10 days at least in the form of Glucantime® administered intramuscularly; and all infections diagnosed were caused by *L. panamensis*. The recurrence rate of leishmaniasis in Opon (3%/year) in the prospective study, was similar to the recurrence rate detected in 59 patients (1.7%/year) in Colombia infected with parasites of the subgenus *L.(Viannia)* treated with the same doses of antimony,

administered intravenously during 20 days in the form of sodium stibogluconate (Pentostam ®) (Saravia & Weigle, 1996).

In previous works, recurrences have been reported from Colombia, Peru and Brazil at rates of 2.0%/year (Weigle et al, 1993), 2.9%/year (Davies et al, 1995a), and 2.7%/year (Jones et al, 1987). The consistency of these data is remarkable given the differences in the treatment, regimes, parasite species, and even in the definition of recurrence used by the different research teams. In the Pacific coast of Colombia (Tumaco), where *L. panamensis* was the most common parasite isolated (Saravia et al, 1990), the group of recurrent patients (80/498) required higher doses of Glucantime and showed a lower MST response compared with non-recurrent patients, suggesting that the risk of reactivation is associated with a low-cell mediated immune response.

In Opon, as in the Peruvian study, the incidence rate of recurrence decreased dramatically during the first 10 years following the primary lesion, indicating that the majority of recurrent leishmaniasis in this period is due mainly to relapses rather than reinfections. Also, in Tumaco the cumulative frequency of recurrences in the group of 498 patients increased rapidly in the first year after the first lesion, but few recurrences were detected during the following 42 months of the follow up (Saravia et al, 1990, 1996). From this group of recurrent cases, 50% were suggested to be caused by relapses

based on the apparent genetic identity of parasites isolated from the same patient in both leishmaniasis episodes (primary and secondary infections) (Saravia et al, 1990). In all patients with identical parasites in consecutive episodes, the secondary lesions were located on the same part of the body on the primary lesions. In the Opon focus, 35% of recurrent lesions were located on the same part of the body as the previous scars, and this percentage decreased significantly with time since primary lesion, indicating that the early recurrences are more likely to be relapses and the later recurrences are more likely to be reinfections (presumably due to the loss of acquired immunity)

4.3.3.2 Acquired immunity

Age was associated with protection against clinical infection in the Opon focus, as it had been in the Tumaco and Peruvian studies. On average, the proportion of infections, carrying clinical symptoms decreased in Opon by 5.4%/year. It is possible that the protective effect of ageing is related to the development of undetected acquired immunity; but it is also possible that the immune system in older people is innately better at coping with leishmaniasis infection. Direct evidence for acquired immunity came from a comparison of clinical infection rates in cohorts defined by their prior clinical status and their MST responsiveness.

In Opon, people with previous clinical episodes (i.e. scars) on average had 80% protection against subsequent clinical infections, although this effect was more marked amongst people who had a negative MST response. MST responsiveness was also a significant indicator of acquired protection, even amongst those with no clinical symptoms. Amongst the healthy population, the odds of a subsequent clinical infection decreased by 22% for each 1 mm increase in MST size in Opon. This corresponds with an equivalent measurement of 18% in a Peruvian study of acquired immunity following subclinical infections. Hence, the Opon results confirm the conclusions from Peruvian study that protective immunity can be acquired following a *Leishmania* infection even in the absence of clinical symptoms. This result provides hope for the future development of vaccines. In addition, it provides a rationale for using the MST response as an indirect indicator of the protection which may follow a putative vaccine within the context of an intervention trial.

5. SANDFLY VECTORS

5.1 INTRODUCTION

The diversity of sandfly fauna in the American Continent is remarkably high compared with the sandfly fauna in the Old World: about 400 species are known in the New World (CIPA group, 1993) but only 10% of them are proven or suspected vectors of leishmaniasis (Killick-Kendrick, 1990). The evidence used for vector incrimination has traditionally been limited to a number of biological criteria (Lewis & Ward, 1987; Killick-Kendrick 1990), but statistical associations can assist in vector incrimination in the absence of other biological evidence (Davies et al 1997b). The biological evidence required for vector incrimination considers the following criteria: (1) presence of the sandfly species in the focus of leishmaniasis transmission; (2) demonstration that the sandfly is anthropophilic and, if the disease is a zoonosis, also feeds on the animal reservoir; (3) isolation of the same *Leishmania* strain from patients and sandflies; and (4) vector competence, i.e. the parasite can develop within the sandfly gut and can be transmitted by bite to a susceptible mammal host. In many endemic leishmaniasis sites in the New World, a large number of sandflies species may fit criteria (1) and (2), and it is difficult

to distinguish which play significant vectorial roles. Field infection rates tend to be low, and so statistical comparisons of infection rates in different species are unlikely to be fruitful. Vector competence studies are also of limited value, as the experimental infection rates with colonised sandflies do not necessarily reflect the natural situation. In contrast, statistical correlations of spatial variability in sandfly abundance and transmission rate (i.e. "the comparative method", sensu Dye, 1992) provide a powerful tool for quantifying the relative vectorial roles of different suspected vectors. Of course, one must always be aware that correlations do not necessarily imply causality, and evidence of natural infections are still required in order to confirm vectorial roles.

Because the significance of risk factors associated with leishmaniasis transmission depends largely on the temporal and spatial patterns of vector abundance, the behaviour and ecology of the suspected sandfly species have been extensively studied in Central America and the Andean countries. However, there is little consistency in the conclusions drawn from the field studies carried out to investigate, for example, sandfly seasonal variation or nocturnal activity in different endemic sites. Because the methodology used in the various sandfly studies has not been standardised, it is also extremely difficult to determine the ecological causes of the different behaviour patterns observed. This task is made more difficult by the incomplete and inconsistent means of reporting entomological data in published papers; e.g.

some report geometric means, others use arithmetic means, and so on. Hence, it is not possible to extrapolate the results from one focus to another, and it remains necessary to conduct entomological studies in each focus under investigation.

The first part of this study focused on the biological evidence for vector incrimination in the Opon focus, i.e. by identifying the anthropophilic sandfly species in this region, and by the detection of *Leishmania* infections in field caught sandflies. Further evidence for vector incrimination, and a quantitative comparison of the vectorial role of the suspected vector species, was provided by a series of regression analyses comparing human transmission rates (incidence and prevalence) with sandfly abundance inside houses. The second part of this study focused on the risk factors for leishmaniasis transmission associated with sandfly abundance and behaviour: i.e. seasonal variation, nocturnal activity, habitat preference and endophagic activity. Thus, the entomological study in Opon focus has as its general objectives:

1. To evaluate the relative vectorial roles of the antropophilic sandfly species present in the Opon focus

2. To identify the risk factors (spatial and temporal) which influence the abundance and distribution of these putative vector species in the Opon focus

The specific objectives were as follows:

1. To identify the anthropophilic sandfly species in the Opon focus
2. To measure the infection rate in the most common anthropophilic species
3. To evaluate the relative vectorial roles of the most common anthropophilic species by searching for spatial associations between sandfly abundance and transmission rate
4. To describe the daily biting cycle, and the seasonal variation of the potential vectors
5. To quantify the effect of habitat (i.e. land use) on the abundance and distribution of the potential vectors, paying particular attention to the effects of deforestation

In addition, this thesis will highlight the necessity of future collaborations between the various sandfly research groups in the Andean Countries which would permit quantitative “meta-analyses” of the sandfly data already collected, in order to provide a clearer idea of the variables determining the distribution and behaviour of all the suspected sandfly vectors in the region.

5.2 RESULTS

5.2.1 Description of the sandfly fauna in the Opon focus

A total of 4,650 adults (4,281 females, 369 males) belonging to 27 sandflies species were caught in the Opon focus between January 1994 - January 1997. Thirty eight sandflies were unidentified due to damage. 96% of the sandflies collected comprised nine species: *Lu. trapidoi* (29.8%), *Lu. hartmanni* (25%), *Lu. quasitowsendi* (22.6%), *Lu. gomezi* (8.2%), *Lu. shannoni* (3.6%), *Lu. camposi* (2.8%), *Lu. ovallesi* (1.5%), *Lu. serrana* (1.4%), and *Lu. yuilli* (1%) (Table 5.1). The species composition of sandfly collections varied by method and by site.

The aim of the entomological studies was not to make direct comparisons of sandfly fauna according to habitat type or trapping method, but to address specific questions about seasonality, nocturnal activity, risk factors for indoor sandfly abundance, and natural infection rate (see below). However, the data do provide some hints about the differences in habitat preference between the sandfly species in this region, and these are presented in sections 5.2.1.1 - 5.2.1.5

Table 5.1 Species composition in the Opon focus

subgenus	species	Shannon trap	free trunks		CDC LIGHT TRAPS		MAN LANDING		total females	total males	TOTAL
			resting	outdoors	indoors	seas.varl.	noct. act.	natural inf.			
NYSSOMYIA	<i>Lu. trapidoi</i>	10	0 (4)*	2	126 (30)	173 (15)	465	558 (4)	1334	53	1387
HELCOCYRTOMYIA	<i>Lu. hartmanni</i>	1	2	1	29 (7)	381 (10)	628	106	1148	17	1165
verrucarum group	<i>Lu. quasitowsendi</i>	17	2 (1)	2 (1)	20 (2)	81	18	905	1046	4	1050
LUTZOMYIA	<i>Lu. gomezi</i>	3	2 (1)	2 (1)	78 (28)	11	92	165	353	30	383
PSATHYROMYIA	<i>Lu. shannoni</i>		26 (121)	2 (9)	4 (1)		4	1	37	131	168
PRESSATIA	<i>Lu. camposi</i>		0 (2)		96 (32)				96	34	130
verrucarum group	<i>Lu. ovallesi</i>	2	6 (7)		40 (5)		2	7	57	12	69
verrucarum group	<i>Lu. serrana</i>		9 (37)		18 (1)				27	38	65
NYSSOMYIA	<i>Lu. yuilli</i>				7	30	11	2	50	0	50
PSYCHODOPYGUS	<i>Lu. panamensis</i>				16	1	3	20	40	0	40
OSWALDOI group	<i>Lu. trinidadensis</i>		2 (14)	1	5 (1)				8	15	23
PINTOMYIA	<i>Lu. christenseni</i>		4 (14)	1					5	14	19
MIGONEI group	<i>Lu. walkeri</i>				12 (2)				12	2	14
COROMYIA	<i>Lu. vespertilionis</i>		1 (13)						1	13	14
MIGONEI group	<i>Lu. dubitans</i>				9 (1)				9	1	10
SAULENSIS group	<i>Lu. saulensis</i>				9				9	0	9
HELCOCYRTOMYIA	<i>Lu. erwindonaldi</i>	1	0 (1)			3			4	1	5
VIANNAMYIA	<i>Lu. tuberculata</i>				2				2	0	2
VIANNAMYIA	<i>Lu. caprina</i>		1						1	0	1
PSATHYROMYIA	<i>Lu. abbonenci</i>		0 (1)						0	1	1
PSATHYROMYIA	<i>Lu. dasymera</i>				1				1	0	1
NYSSOMYIA	<i>Lu. olmeca bic.</i>		0 (1)						0	1	1
MICROPYGOMYIA	<i>Lu. yencanensis</i>		0 (1)						0	1	1
MICROPYGOMYIA	<i>Lu. venezuelensis</i>							1	1	0	1
LUTZOMYIA	<i>Lu. bifoliata</i>				1				1	0	1
Genus: Brumptomyia	<i>Br. galindoi</i>		0 (1)						0	1	1
	Subg. tricopigomyia				1				1	0	1
	unidentified							38	38		38
	TOTAL	34	55 (219)	11 (11)	475 (110)	680 (25)	1223 (0)	1803 (4)	4281	369	4650

* female (male)

5.2.1.1 Man-landing collections

a) Seasonal variation study

Sandflies were collected from 18:00 to 20:00 hours at intervals throughout the year at eight sites in San Pedro, representing four habitat types: (1) indoors, (2) in the peri-domicile, (3) in cacao, and (4) in the forest (four sites located in/or around House 1, and four sites in/or around House 2). The species composition was consistent between pairs of sites representing similar habitats, but differed between habitat types. (Table 5.2)

Table 5.2 Sandfly abundance measured by man-landing collections in four habitat types, two sites/habitat and 18 collections/site (18:00-20:00 hours): Seasonal variation study in San Pedro

	inside house		peridomestic		cacao crops		forest		TOTAL
	n	mean*	n	mean*	n	mean*	n	mean*	
<i>Lu. trapidoi</i>	17	17.2	40	64.1	47	65.5	69	55.3	173
<i>Lu. gomezi</i>	1	1.90	5	9.2	4	7.1	1	1.9	11
<i>Lu. hartmanni</i>	7	11.3	102	128.4	110	169.9	162	156.9	381
<i>Lu. yuilli</i>	0	0	10	17	14	15.4	6	8.6	30
<i>Lu. quasitowsendi</i>	20	22.3	15	24.4	17	27.1	29	34.7	81
<i>Lu. panamensis</i>	0	0	1	1.9	0	0	0	0	1
<i>Lu. erwindonaldi</i>	0	0	0	0	3	5.1	0	0	3

* = geometric mean of 36 replicates (number/100 man-hours)

Lu. hartmanni predominated in all habitat types with the exception of inside the house, where it was under-represented: the mean landing rate indoors was only 9% that in the peridomestic area, indicating very low endophagic activity. *Lu. trapidoi* was the second most common species in all habitats, but again was relative rare indoors: the mean indoor landing rate was 27% that in the peridomicile. In contrast *Lu. quasitowsendi* showed a relatively high level of endophagy, with no significant difference between the landing rate indoors and outdoors (significantly greater than both *Lu. trapidoi*, $\chi^2 = 8.64$; $P < 0.01$ and *Lu. hartmanni* , $\chi^2 = 59.32$; $P < 0.001$). The numbers collected for the remaining species were too low to draw firm conclusions. Comparing the three outdoor habitats, there is a slight suggestion that *Lu. quasitowsendi* is relatively common in the forest, and that *Lu. hartmanni* is relatively rare in the peridomicile. The abundance of *Lu. trapidoi* was relatively constant in all three outdoor habitat types sampled. Additionally, there was some suggestion that *Lu. gomezi* was underrepresented in the forest since 10% (1/10) individuals sampled outdoors were collected there, whilst the proportion of the other anthropophilic species in the forest was significantly higher : 44% (69/156) for *Lu. trapidoi* (Yates corrected $\chi^2 = 3.22$; $P = 0.03$) ; 43% (162/374) for *Lu. hartmanni* (Yates corrected $\chi^2 = 3.17$; $P = 0.03$); and 47% (29/61) for *Lu. quasitowsendi* (Yates corrected $\chi^2 = 3.54$; $P = 0.02$) (Table 5.2).

b) Nocturnal activity study

Sandflies were collected from 18:00 to 06:00 hours at four sites representing four habitats (1site/habitat) during three days in June 1996, in/or around House 3 located at Santa Sofia: (1) indoors, (2) in the peridomicile, (3) in the cacao and (4) in the forest. The forest around House 3 was less disturbed by human activity than the forest collection sites around Houses 1 and 2 in San Pedro, and the cacao crops were younger than those in San Pedro. The total number of sandflies collected in the nocturnal activity study was almost double that in the seasonal variation study, even though the total number of man-landing collection hours man was exactly half that of the seasonal variation study (Table 5.3). The species compositions of the collections from the two studies were similar except that *Lu. ovallesi* and *Lu. shannoni* were collected only in the nocturnal study, and *Lu. erwindonaldi* was collected only in the seasonal study (Table 5.3).

Lu. hartmanni was the predominant species in all habitats with the exception of the forest; its endophagic activity in House 3 was considerably higher than that detected in Houses 1 and 2 in the seasonal variation study, with a mean man-landing rate equivalent to 40% that in the peri-domicile. *Lu. trapidoi* was the most common species in the forest and the second most commonly collected in the other three habitats sampled. The *Lu. trapidoi* collections in the domestic habitat were too small to draw any firm conclusion

concerning endophagic activity, although there was some evidence suggesting that, as for *Lu. hartmanni*, the endophagic activity of *Lu. trapidoi* was higher here than in Houses 1 and 2 in San Pedro.

Table 5.3 Sandfly abundance measured by man-landing collections in four habitat types, one site/habitat and three collections/site (18:00-06:00 hours): Nocturnal activity study in Santa Sofia

species	inside house		peridomestic		cacao crops		forest		TOTAL
	n	mean*	n	mean*	n	mean*	n	mean*	
<i>Lu. trapidoi</i>	6	6.5	2	3.6	170	210.8	287	455.8	469
<i>Lu. gomezi</i>	2	3.6	1	1.7	54	78.2	35	53.8	92
<i>Lu. hartmanni</i>	6	9.6	13	24.1	304	441.4	305	343.2	628
<i>Lu. yuilli</i>	2	3.6	0	0	5	7.3	4	7.3	11
<i>Lu. quasitowsendi</i>	1	1.7	0	0	1	1.7	16	28.6	18
<i>Lu. panamensis</i>	0	0	0	0	3	5.4	0	0	3
<i>Lu. ovallesi</i>	0	0	0	0	0	0	2	3.6	2
<i>Lu. shannoni</i>	0	0	0	0	0	0	4	5.4	4

* = geometric mean of 3 replicas (number/100 man-hours)

Unlike in the seasonal study, *Lu. gomezi* was well represented in the nocturnal study, being the third most important species in all four habitat types sampled. Another contrast with the seasonal variation study was demonstrated by *Lu. quasitowsendi*, which was abundant in the forest but rarely encountered in the other habitat types. As before, the numbers collected for the remaining species were too low to draw firm conclusions. Comparing the three outdoor habitats, there is some suggestion that *Lu.*

hartmanni and *Lu. gomezi* were relatively common in the cacao, whereas *Lu. trapidoi* and *Lu. quasitowsendi* were relatively common in the forest. All four species were relatively rare in the peri-domicile. As in the seasonal variation study, *Lu. gomezi* was underrepresented in the forest collections : 39% (35/90) *Lu. gomezi* compared with 62% (287/459) *Lu. trapidoi* (Yates corrected $\chi^2 = 16.3$; $P < 0.001$) ; and 94% (16/17) *Lu. quasitowsendi* (Yates corrected $\chi^2 = 15.34$; $P < 0.001$). However, differences with *Lu. hartmanni* were not significant 49% (305/622) (Yates corrected $\chi^2 = 2.85$; $P = 0.09$) (Table 5.3).

c) Natural infection study

Natural infections were sought in 1,803 sandflies from man-landing collections (on three nights) in Forest 4 in the village of San Pedro, 15 Km north-west of Houses 1 and 2. The collection was dominated by *Lu. quasitowsendi* (50.2%) followed by *Lu. trapidoi* (31%), *Lu. gomezi* (9.1%) and *Lu. hartmanni* (5.8%). A few individuals of *Lu. panamensis*, *Lu. ovallesi*, *Lu. yuilli*, *Lu. shannoni* and *Lu. venezuelensis* comprised the remainder of the collections (Table 5.1). Thirty eight sandflies were not identified to species due to damage.

The main contrasts between the sandfly catch at Forest 4 compared to the forest catches around Houses 1, 2 and 3 were the relatively high

abundance of *Lu. quasitowsendi* and *Lu. panamensis*, and the relatively low abundance of *Lu. hartmanni*. Forest 4 was also the only site in the whole project where *Lu. venezuelensis* was collected.

5.2.1.2 Shannon trap

In the pilot study in February 1994 (1 night), six anthropophilic species were identified amongst the 34 sandflies collected in the peri-domicile around House 5 located in the village of La Soledad. In contrast to the catches in the peri-domicile of Houses 1,2 and 3, *Lu. hartmanni* was relatively rare and *Lu. quasitowsendi* was relatively abundant (50% of the total catch). This was also the only peridomestic site in the project where *Lu. erwindonaldoi* and *Lu. ovallesi* were found.

5.2.1.3 CDC light traps

A total of 585 sandflies, comprising 19 *Lutzomyia* species, were collected from 18:00 to 06:00 hours inside 114 houses in 11 villages (one trap-night/house) in February 1996 (during the study of risk factors for indoor sandfly abundance). These included eight anthropophilic species (i.e. species also collected at least once in the man-landing catches). *Lu. trapidoi* was the most abundant species (geometric mean: 0.48 /trap-night) followed by *Lu. gomezi* (0.38 /trap-night), *Lu. ovallesi* (0.19 /trap-night) and *Lu.*

hartmanni (0.14 /trap-night). The sex ratio for all 19 species was highly female biased, with the percentage of females within the total catch ranging from 74% for *Lu. gomezi* to 100% for seven of the rarer species. However, the sex ratio of each species never varied significantly from 81%, the overall mean ($\chi^2 = 6.70$; $P > 0.05$; D.F. = 11).

A direct comparison of the sandfly fauna in CDC and man-landing catches can be made by focusing on the results from the villages of San Pedro and Santa Sofia (Table 5.4). The species diversity in the CDC light trap collections was clearly higher: 10 species were identified amongst the 42 sandflies collected compared to five species amongst the 62 sandflies in the man-landing collections. Amongst the anthropophilic species, the main differences between the two trapping methods were a relatively low representation of *Lu. hartmanni* and *Lu. quasitowsendi* in the CDC light traps collections (Yates corrected $\chi^2 = 11.8$; $p < 0.001$) and a relatively low representation of *Lu. gomezi* in the man-landing catches collections (Yates corrected $\chi^2 = 12.8$; $p < 0.001$). The anthropophilic and phototropic behaviour of the main two species in the Opon focus (*Lu. gomezi* and *Lu. trapidoi*) can be compared by measuring the ratio of the abundance of these two species in each trap method: the proportion of *Lu. gomezi* (80/128, 38%) caught in light traps was significantly greater than the proportion caught in man-landing

catches (268/1,196, 22%) ($\chi^2 = 45.16$; $p < 0.001$; $RR = 2.1$; 95% C.I., 1.74 - 2.61).

Table 5.4 Indoor collections of sandflies using CDC light traps in 13 villages (114 night - traps)

SPECIES	TOTAL		SP + SS ^a	
	n	mean*	n	mean+
<i>Lu. trapidoi</i> •	126	47.7	13	41.4
<i>Lu. gomezi</i> •	78	38	14	46.1
<i>Lu. ovallesi</i> •	40	19.1	1	4.4
<i>Lu. hartmanni</i> •	29	14.1	2	9
<i>Lu. camposi</i>	96	8.9	1	4.4
<i>Lu. serrana</i>	18	8.7	2	9
<i>Lu. panamensis</i> •	16	8.5	1	4.4
<i>Lu. quasitowsendi</i> •	21	8.1	6	21.9
<i>Lu. walkeri</i>	12	5.4		
<i>Lu. saulensis</i>	9	4.9	1	4.4
<i>Lu. dubitans</i>	9	4.4		
<i>Lu. yuilli</i> •	7	3.6	1	4.4
<i>Lu. trinidadensis</i>	5	2.7		
<i>Lu. shannoni</i> •	4	2.4		
<i>Lu. tuberculata</i>	2	0.9		
Subgenus <i>tricopigomyia</i>	1	0.6		
<i>Lu. bifoliata</i>	1	0.6		
<i>Lu. dasymera</i>	1	0.6		
Total	475		42	

* geometric mean of 114 /100 trap-nights

+ geometric mean/100 man-hour (see Tables 5.2 and 5.3)

a = collections in San Pedro and Santa Sofia

• anthropophilic species

5.2.1.4 Resting places: tree trunks

In the pilot study, 219 sandflies representing 17 species were caught during the day by aspiration from trees located in the forest, cacao and peri-domicile in the village of La Soledad. Six species in this catch were absent in both the man-landing and CDC light trap collections: *Lu. vespertilionis*, *Lu. caprina*, *Lu. abonnenci*, *Lu. olmeca bicolor*, *Lu. yencanensis* and *Br. galindoi*. Seven out of the ten anthropophilic species in the Opon focus were found resting on tree trunks; the exceptions were *Lu. yuilli*, *Lu. panamensis* and *Lu. venezuelensis*. The main contrasts between the sandfly collections from the resting places compared to the outdoor man-landing catches were the relatively low abundance of the four main anthropophilic species (*Lu. trapidoi*, *Lu. hartmanni*, *Lu. quasitowsendi*, and *Lu. gomezi*), and the relatively high abundance of *Lu. shannoni* (54% of the total resting site collection). Males were more abundant than females: the mean percentage of females within the total catch of the 17 species did not differ significantly from 20.1%, the overall mean ($\chi^2 = 6.70$; $P > 0.05$; D.F. = 14).

5.2.1.5 Additional collections in the region

During the pilot study in February 1994, CDC light traps collection were also made during one night in the peri-domicile of House 5 in La Soledad. Seven species were identified amongst the 22 sandflies collected

(Table 5.1). The main contrast with the indoor CDC catches (in house 5 in La Soledad) were the predominance of *Lu. shannoni* (50% of the total catch), and the detection of *Lu. christenseni*, which was never found in any of the 114 houses sampled.

5.2.2 Natural infection of sandflies

A total of 1,803 sandflies were dissected in the search for *Leishmania* promastigotes in the digestive tract. Of those dissected, 11 *Lu. trapidoi* (2%), 7 *Lu. quasitowsendi* (0.8%), and 3 *Lu. gomezi* (1.8%) were found with flagellates (Table 5.5). Both hamster inoculation (for attempted *in vivo* isolation) and direct PCR were carried out on each flagellate positive sandfly. All hamsters were negative after 4 months of observation, and only 2 *Lu. trapidoi* were positive by PCR using a *Leishmania braziliensis* complex specific primer. The parasites could not be identified to species because the DNA probe used for hybridisation of PCR products could not distinguish between species in the *L. braziliensis* complex (i.e. the probe reacted with both *L. braziliensis* and *L. panamensis* reference strains, acting as controls on the gel). However, based on the characterisation of parasites isolated from patients in Opon (see Chapter 3), and on previous studies of natural infections in *Lu. trapidoi*, it is most likely that the parasites detected in *Lu. trapidoi* were *L. panamensis*.

Table 5.5 Natural infection of sandflies in the Opon focus

sandfly spp.	No. dissected	(+) promast.	%	PCR +	%
<i>Lu. trapidoi</i>	558	11	2	2	0.35
<i>Lu. quasilowsendi</i>	905	7	0.8	0	0
<i>Lu. gomezi</i>	165	3	1.8	0	0
<i>Lu. hartmanni</i>	106	0	0	0	0
<i>Lu. panamensis</i>	20	0	0	0	0
<i>Lu. ovallesi</i>	7	0	0	0	0
<i>Lu. yuilli</i>	2	0	0	0	0
<i>Lu. venezuelensis</i>	1	0	0	0	0
<i>Lu. shannoni</i>	1	0	0	0	0
<i>Lutzomyia spp.</i>	38	0	0	0	0
TOTAL	1,803	21	1.2	2	0.11

5.2.3 Nocturnal activity

A total of 1223 female sandflies belonging to 8 species, were captured by man-landing between 18:00 - 06:00 hours at 4 sites (3 nights/site): indoors (n = 17), in the peri-domicile (n= 16), in cacao (n = 538), and in forest (n = 654). *Lu. hartmanni* (n = 628) and *Lu. trapidoi* (n = 465) accounted for 89% of the sandfly collection; and the only other species caught in significant numbers was *Lu. gomezi* (n = 92). The remaining species were *Lu.*

quasitowsendi, *Lu. yuilli*, *Lu. shannoni*, *Lu. panamensis* and *Lu. ovallesi* (Table 5.1).

Of the five most common species collected in the nocturnal study, *Lu. yuilli* was relatively active soon after dark with peak biting activity between 20:00-21:00 hours, and no biting activity detected after 23:00 hours (Figure 5.1 and Figure 5.6). Biting activity of *Lu. hartmanni* also peaked between 20:00-21:00 hours (Figure 5.2), and the median time-point (i.e. the time at which the cumulative biting activity reached 50% of the total during the night) was between 21:00-22:00 hours (Figure 5.6). Peak biting activity for *Lu. gomezi* was especially early, from 19:00-20:00 hours (Figure 5.3) but this species remained active throughout the night at a relatively high rate, with a median time-point between 22:00-23:00 hours (Figure 5.6). *Lu. quasitowsendi* had a similar activity pattern to *Lu. gomezi* with a slight peak between 22:00-23:00 (Figure 5.4 and Figure 5.6). Finally, the activity pattern of *Lu. trapidoi* was quite distinct from the other species, in that it peaked during the second half of the night, between 02:00-03:00 hours (Figure 5.5), and the median time-point was between 01:00-02:00 hours (Figure 5.6).

Figure 5.1 Nocturnal activity of *Lu. yuilli*

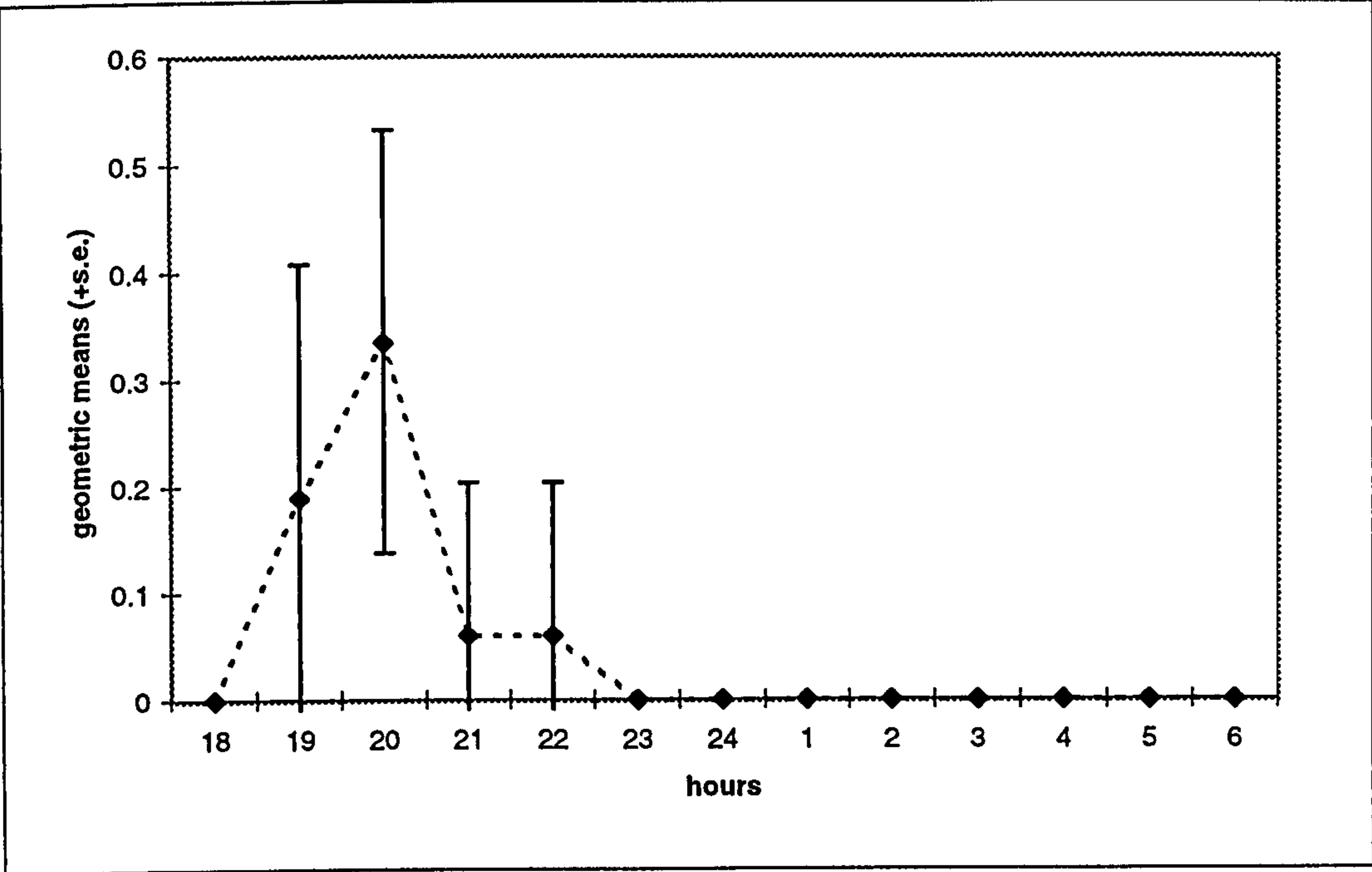


Figure 5.2 Nocturnal activity of *Lu. hartmanni*

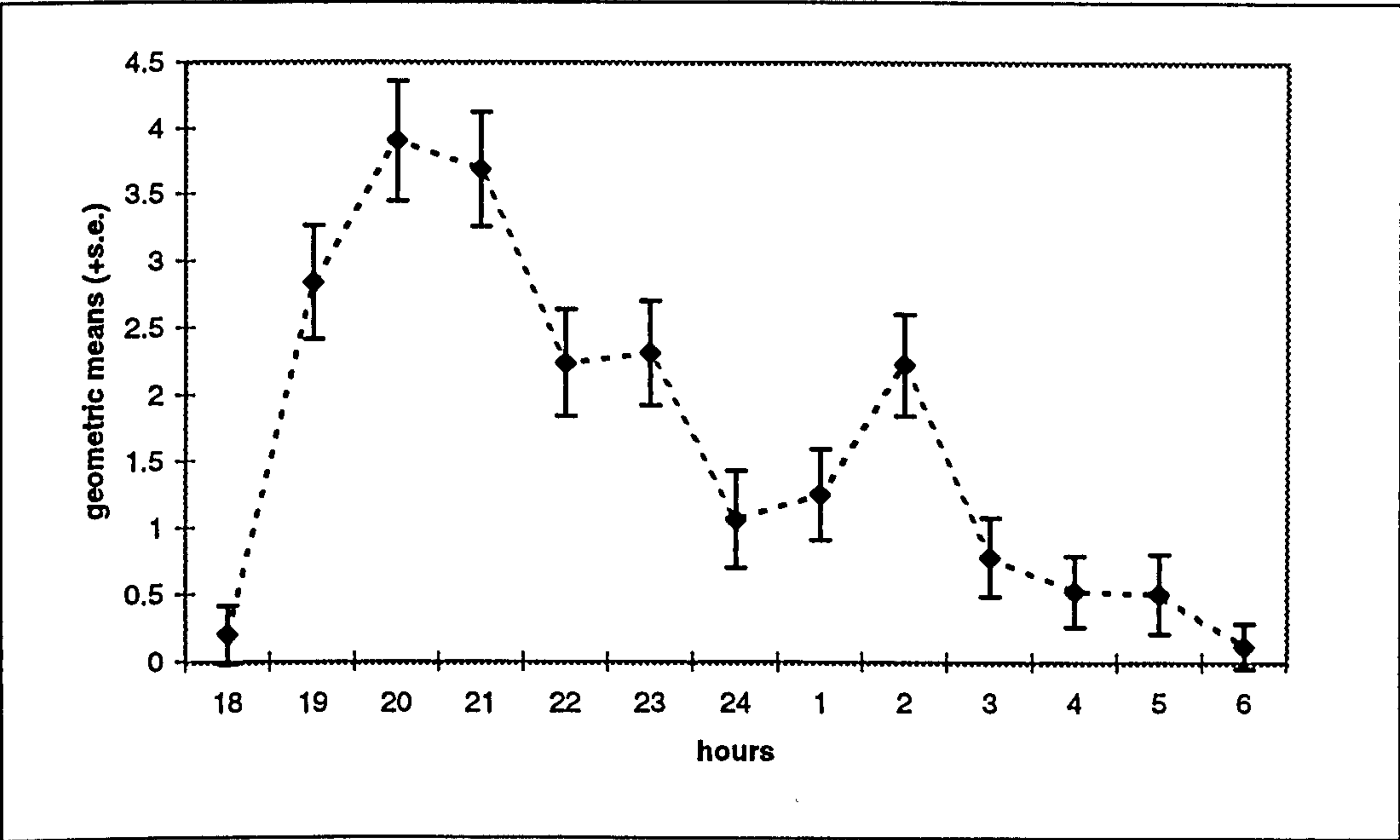


Figure 5.3 Nocturnal activity of *Lu. gomezi*

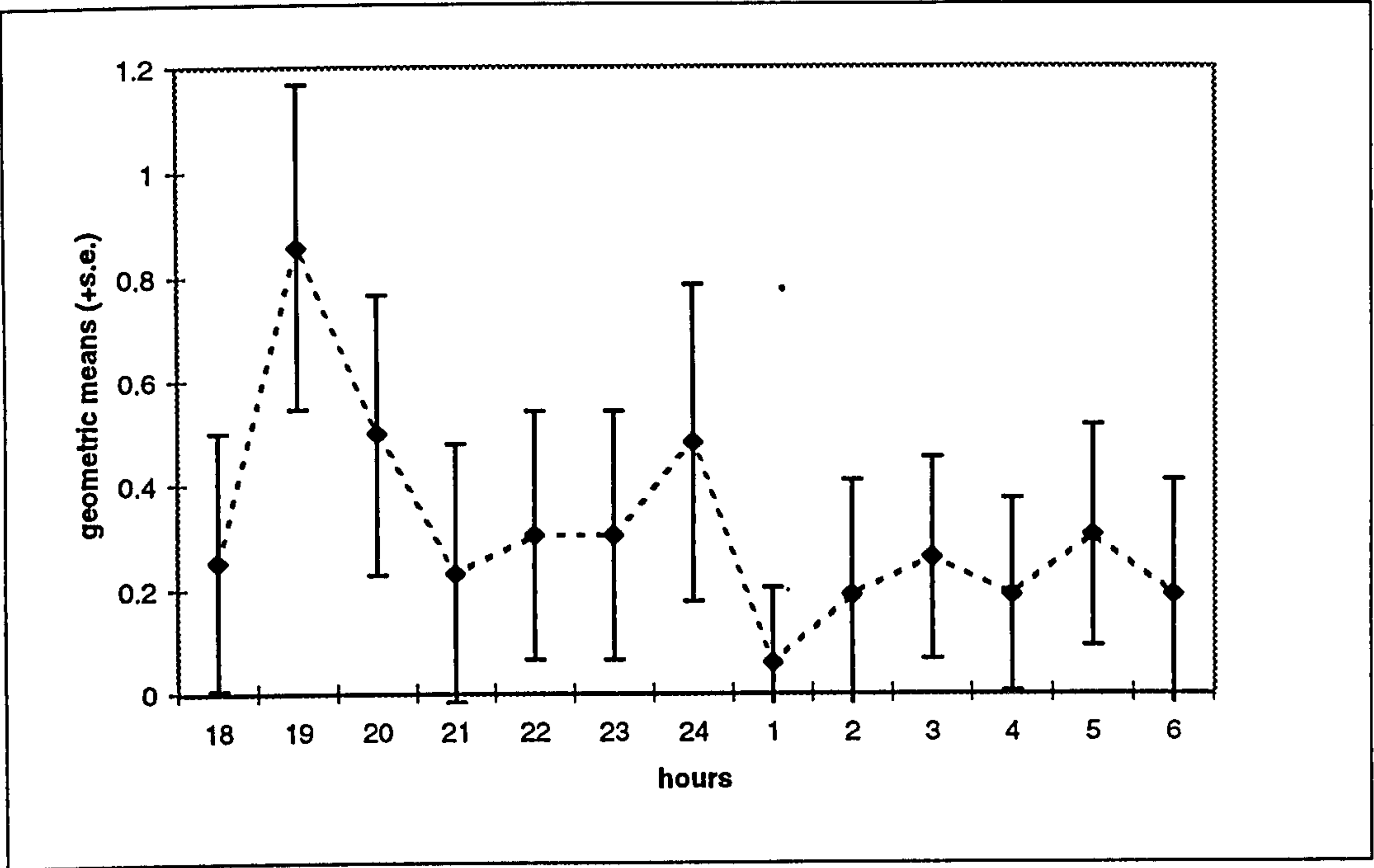


Figure 5.4 Nocturnal activity of *Lu. quasitowsendi*

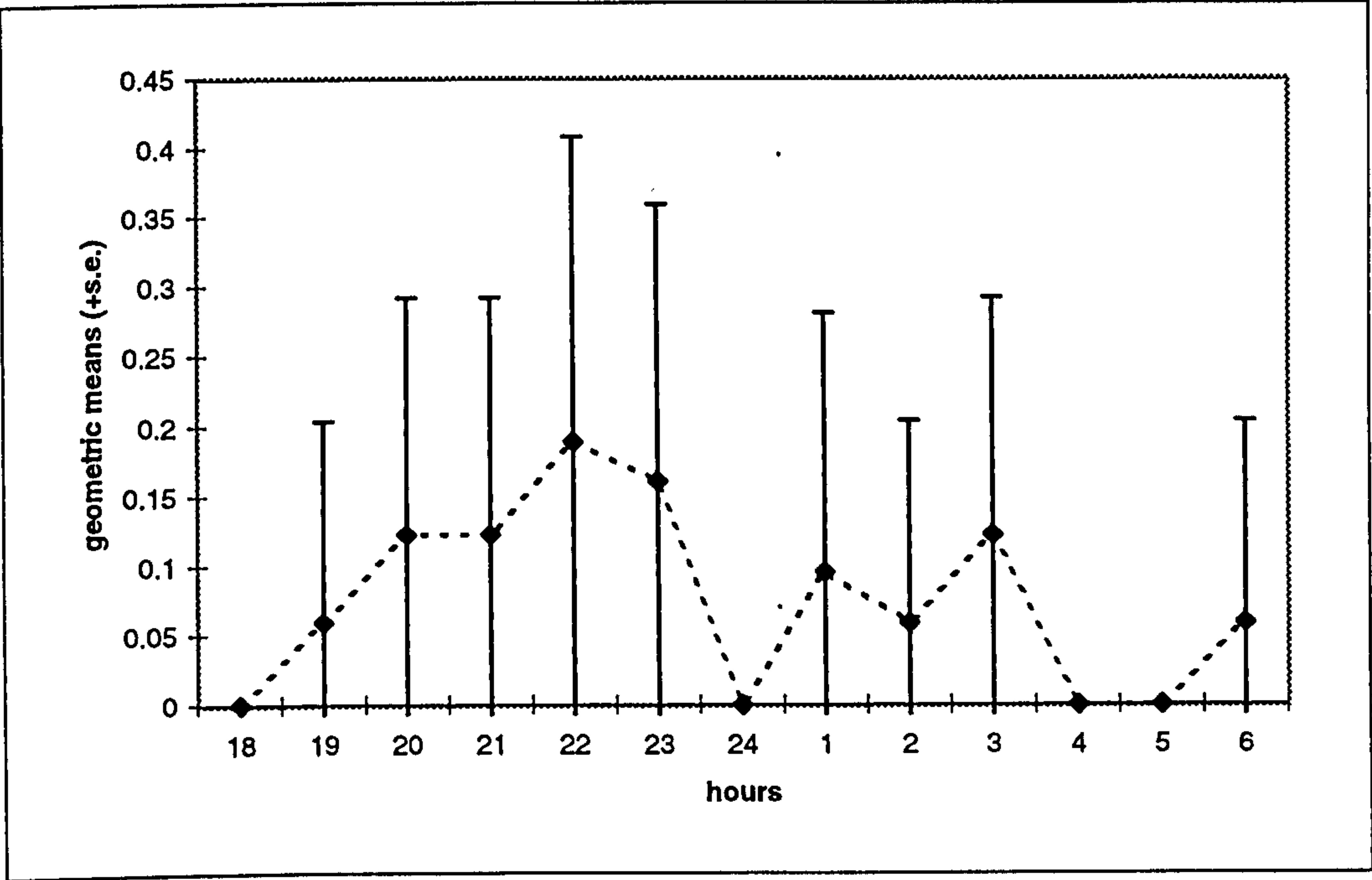
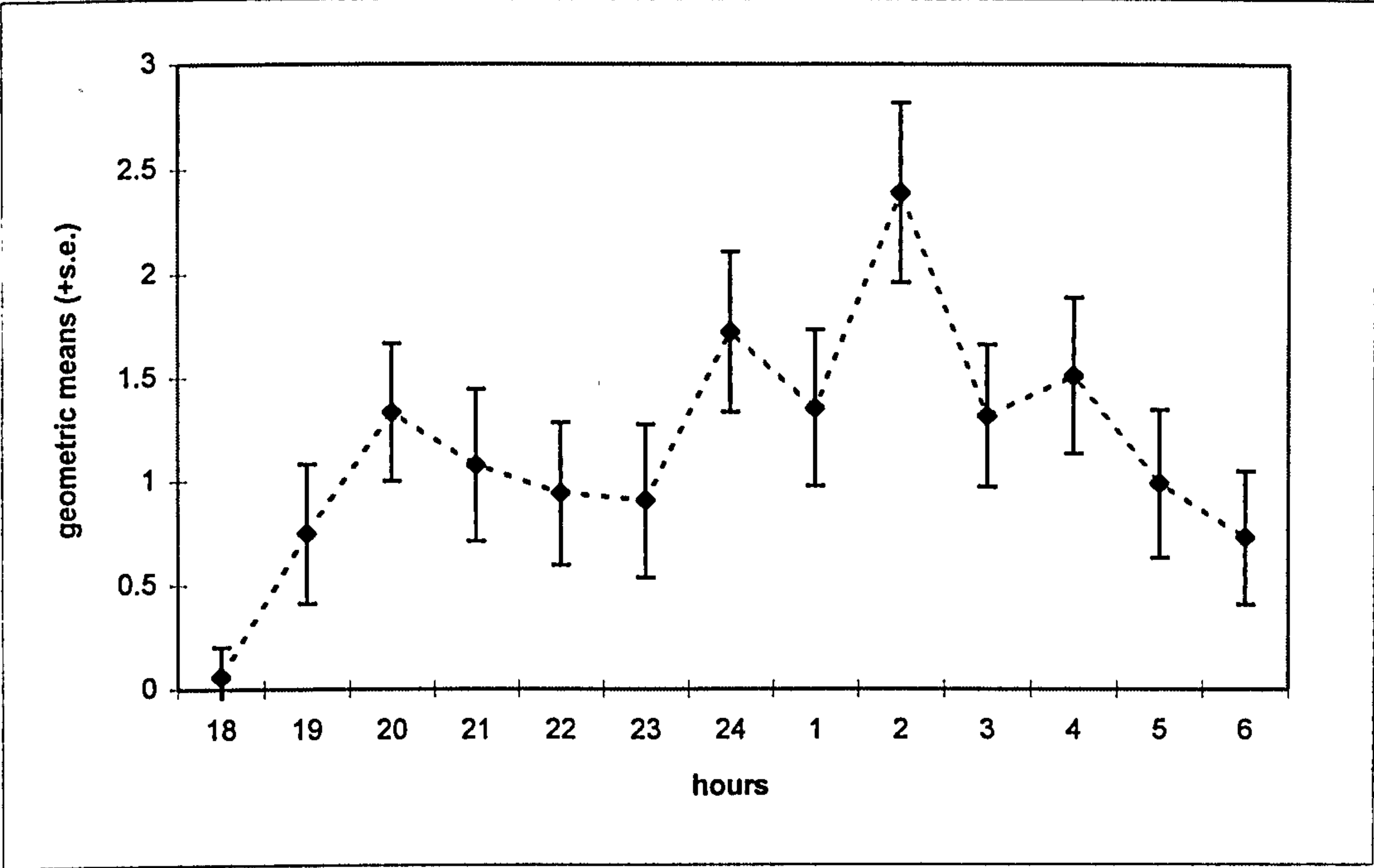
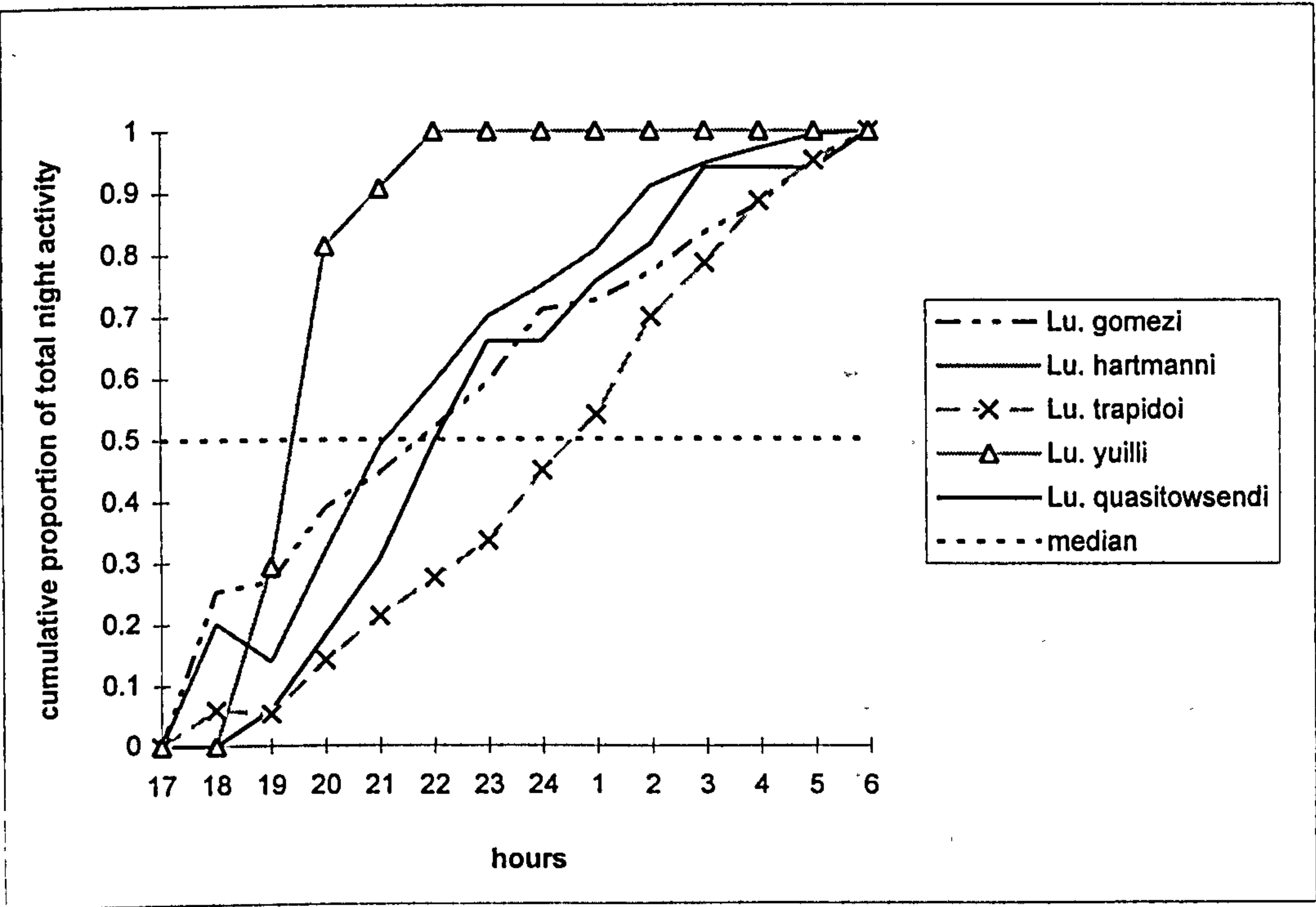


Figure 5.5 Nocturnal activity of *Lu. trapidoi*



Legend Figure 5.1-5,5. Dots represent geometric mean (n = 3) and bars represent the standard errors.

Figure 5.6 Cumulative biting of sandflies during the night (as a proportion of the total night's activity)



122

A statistical comparison of the activity pattern of the five species was made by dividing the night into two sections: (1) early night (18:00-22:00), i.e. before bed time; and (2) mid and late night (22:00-07:00). Chi square test (or Fisher exact test, when appropriated) were carried out on the total catch for each of the five most common species during these two periods, in order to compare the proportion of the biting activity of each sandfly species before bedtime. The proportion of *Lu. trapidoi* biting after 22 :00 hours was significantly greater than that for all three 'early peak' species (*Lu. gomezi*, *Lu. hartmanni* and *Lu. yuilli*) at $p<0.001$. There was also some suggestion that *Lu. yuilli* bites earlier than all the other species ($p< 0.01$ for all species), and some evidence that *Lu. quasitowsendi* bites later than *Lu. hartmanni* ; but there was no difference between the biting activity of *Lu. gomezi* compared with either *Lu. hartmanni* and *Lu. quasitowsendi*. There was also no significant difference between the biting activity of *Lu. quasitowsendi* and *Lu. trapidoi* ($P> 0.05$) (Table 5.6).

Table 5.6 Comparisons between total nocturnal biting activity of sandflies: before and after “bed-time” (22:00 hours)

Time (hrs)	<i>Lu. gome.</i>	<i>Lu. hartman.</i>	<i>Lu. trapidoi</i>	<i>Lu. yuilli</i>	<i>Lu. quasit.</i>
18 - 22	43	334	92	10	5
22 - 07	49	294	373	1	13
total	92	628	465	11	18
P < 0.05	a c*	a	b e	d	c e
P < 0.01	a	a	b c	d	a c
P< 0.0001	a c	a c	b d	a	c d

* A significant difference at the P value shown was demonstrated between those species which do not share the same letter

5.2.4 The effect of vegetation on variability in indoor sandfly abundance

5.2.4.1 A description of the sandfly database

A total of 585 sandflies comprising 19 species, were collected (February 1996) indoors in 114 random selected houses in 11 villages (one trap-night/house) (see Section 5.2.1.3). In this analysis, La Soledad was counted as two villages, as the houses are dispersed over a large area and they divide naturally into two groups of houses separated by a mountain (Figure 2.2). The number of sampled houses per village varied from 7 to 10: 10 houses/village in six villages (including the two parts of La Soledad), nine houses/village in four villages, and seven houses/village in one village (Table 5.7). Valparaiso village was excluded from this study.

Sandflies were collected from 73/114 houses distributed in all 11 sampled villages, with clear differences between villages in the proportion of houses containing sandflies: in La Soledad and Yolandas, for example, where cacao plantations are abundant, sandflies were collected from almost all houses; whereas in Tagual and La Dorada, where there is a high density of pasture land and cattle farms, sandflies were collected from less than half of the houses (see Material and Methods) (Table 5.7). The most abundant species were *Lu. trapidoi* and *Lu. gomezi* (Table 5.1).

Table 5.7 The proportion of houses containing sandflies according to villages

VILLAGES	# of houses of the study pop.	# of houses with sandfly data		# of houses with sandflies collected	
		n	%	n	%
BA	30	9	30	5	56
CU	19	10	53	4	40
DL	19	10	53	5	50
LD	18	9	50	3	33
LS NORTH	11	11	100	11	100
LS SOUTH	38	10	26	10	100
MR	40	9	23	7	78
PA	27	9	33	6	67
SF	14	7	50	5	71
SP	45	10	22	4	40
TG	35	10	29	4	40
VP	19	0	0	0	0
YO	16	10	63	9	90
TOTAL	331	114	34	73	64

Table 5.8 Distribution of sandfly species by village

VILLAGES	altitude (masl)	Lu. trap.	Lu. gom.	Lu. oval.	Lu. quas.	Lu. pana.	Lu. hartm.	other Lut.	Total
BA	600 - 1000	23	5	1	0	0	1	4	34
CU	600 - 1200	2	4	0	0	0	0	4	10
DL	400 - 800	3	4	0	0	1	0	1	9
LD	400 - 600	4	3	2	0	0	0	1	10
LS-east	400 - 800	42	9	15	2	5	9	27	109
LS-west	600 - 800	17	9	10	4	1	5	97	143
MR	800 - 1000	12	3	2	6	1	8	8	40
PA	400 - 600	1	12	2	0	1	0	9	25
SF	400 - 1200	6	15	4	6	1	2	4	38
SP	400 - 1000	9	2	0	0	0	0	0	11
TG	400 - 1000	1	3	0	0	1	0	1	6
YO	500 - 800	6	4	2	2	5	4	12	35
Grand Total		126	73	38	20	16	29	150	470

The analyses described below were carried out only on those sandfly species which have been shown to be anthropophilic in the Opon focus (i.e. they were collected at least once in a man-landing catch or in a Shannon trap), as these species have potential vectorial roles: *Lu. trapidoi*, *Lu. gomezi*, *Lu. ovallesi*, *Lu. hartmanni*, *Lu. panamensis*, and *Lu. quasitowsendi* (Table 5.8)

Although in the Opon focus, altitude varies from 400 to 1200 m a.s.l. (Figure 2.2), the majority of houses are located within an altitude of 600 to 800 m. Because the 114 houses sampled were all located within a narrow altitude range, it was not possible to test altitude as an explanatory variable for sandfly distribution.

Section 5.2.4.2 describes the results of a multiple regression analysis which examines the relationship between the vegetation surrounding a house and the abundance of each sandflies species within that house. Section 5.2.5.1 describes the results of a multiple regression analysis which examines the relationship between the number of each sandfly species collected inside a house and either the incidence rate or prevalence of leishmaniasis within that household. Finally, in section 5.2.5.2, a further multiple regression analysis is carried out to determine the relationship between the geometric mean abundance of each sandfly species in a village and either the village incidence rate or prevalence of leishmaniasis.

5.2.4.2 The relationship between the sandfly fauna in a house and its surrounding vegetation

A multiple regression analysis was carried out for each of the seven anthropophilic species, having as explanatory variables the relative coverage of different vegetation types at distances of 50 m, 100 m, 200 m, 300 m, and 800 m from each of the 114 houses examined. From 35 possible sandfly-vegetation associations, 11 significant predictors were detected (Table 5.9). Cacao crops were the most consistent predictor of intradomiciliary sandfly activity. As cacao coverage around houses increased, there was a significant increase in the indoor abundance of *Lu. trapidoi*, *Lu. gomezi* and *Lu. ovallesi*. For *Lu. trapidoi* this effect increased with distance from the house, reaching a peak association with vegetation coverage up to 300 m. The “300 m model”, which explains 25% of the variance in *Lu. trapidoi* indoor abundance, predicts that an increase in 1% in the coverage with cacao up to 300 m from a house, should be associated with an increase of 4% in *Lu. trapidoi* indoor abundance ($e^{1 \times 0.04}$). Similarly, the effect of cacao coverage on *Lu. ovallesi* abundance becomes more marked with distances up to 800 m from the house. The “800 m model”, which explains 24% of the variance in indoor *Lu. ovallesi* abundance, predicts that an increase in 1% in the coverage with cacao up to 800 m, is associated with an increase of 6% in the *Lu. ovallesi* indoor abundance. In contrast, the relatively weak positive relationship between cacao coverage and *Lu. gomezi* indoor abundance was

only detected for vegetation indices up to 100 m from a house. Weak negative relationships were also detected between the indoor abundance of *Lu. trapidoi* and *Lu. ovallesi* and the amount of surrounding pasture and secondary forest respectively.

Negative relationships between pasture land and the abundance of all three sandflies species were exposed by a series of univariate analysis (Table 5.10), but these effects dropped out of multivariate model due to a significant negative correlation between the relative amounts of pasture and cacao surrounding the 114 houses.

Table 5.9 Sandfly activity inside houses according to surrounding vegetation type: Parameter estimates, standard errors and r^2 for the minimal adequate models generated by multiple regression analyses

	50 m.		100 m.		200 m.		300 m.		800 m.	
<i>Lu. trapidoi</i>										
INTERCEPT	0.846	*	-0.562	**	-0.775	***	-1.027	**	-	
(S.E)	(0.332)		(0.329)		0.346		(0.363)		-	
SLOPE	-0.017		0.025		0.032		0.041		-	
(S.E)	(0.007)		(0.007)		(0.008)		(0.008)		-	
r ²	0.096		0.130		0.170		0.250		-	
parameter	pasture		cacao		cacao		cacao			
<i>Lu. gomezi</i>										
INTERCEPT	-0.897	*	-0.918	*	-		-		-	
(S.E)	(0.307)		(0.316)		-		-		-	
SLOPE	0.014		0.019		-		-		-	
(S.E)	(0.006)		(0.008)		-		-		-	
r ²	0.074		0.070		-		-		-	
parameter	cacao		cacao		-		-		-	
<i>Lu. ovallesi</i>										
INTERCEPT	-1.965	***	-0.385	*	-2.037	**	-2.190	***	-2.365	***
(S.E)	(0.408)		(0.371)		(0.437)		(0.458)		(0.517)	
SLOPE	0.023		-0.043		0.034		0.040		0.054	
(S.E)	(0.007)		(0.021)		(0.010)		(0.010)		(0.015)	
r ²	0.160		0.080		0.160		0.200		0.240	
parameter	cacao		sec. forest		cacao		cacao		cacao	

(S.E.) = Standard Error

*** P<0.001, ** P<0.01, * P<0.05

Table 5.10 Sandfly activity inside houses according to the extent of surrounding pasture land: Parameter estimates, standard errors and r^2 for the minimal adequate models generated by univariate regression analyses

	50 m.		100 m.		200 m.		300 m.		800 m.	
<i>Lu. trapidoi</i>										
INTERCEPT	0.846	*	0.958	*	1.029	*	1.174	**	-	
(S.E)	(0.332)		(0.370)		(0.377)		(0.346)		-	
SLOPE	-0.017		-0.020		-0.024		-0.031			
(S.E)	(0.007)		(0.008)		(0.010)		(0.010)			
r2	0.096		0.086		0.091		0.140			
<i>Lu. gomezi</i>										
INTERCEPT	0.134	*	-		-		-		-	
(S.E)	(0.313)									
SLOPE	-0.012		-		-		-		-	
(S.E)	(0.006)									
r2	0.059		-		-		-		-	
<i>Lu. ovallesi</i>										
INTERCEPT	-0.280	**	-0.208	*	0.024	**	0.100	**	-0.228	*
(S.E)	(0.327)		(0.395)		(0.396)		(0.381)		(0.381)	
SLOPE	-0.019		-0.021		-0.031		-0.036		-0.030	
(S.E)	(0.007)		(0.009)		(0.011)		(0.012)		(0.013)	
r2	0.099		0.077		0.120		0.090		0.054	

(S.E.) = Standard Error
** P < 0.01, * P < 0.05

5.2.5 The relationship between indoor sandfly abundance and transmission rate.

5.2.5.1 Variation at the household level

a) Incidence

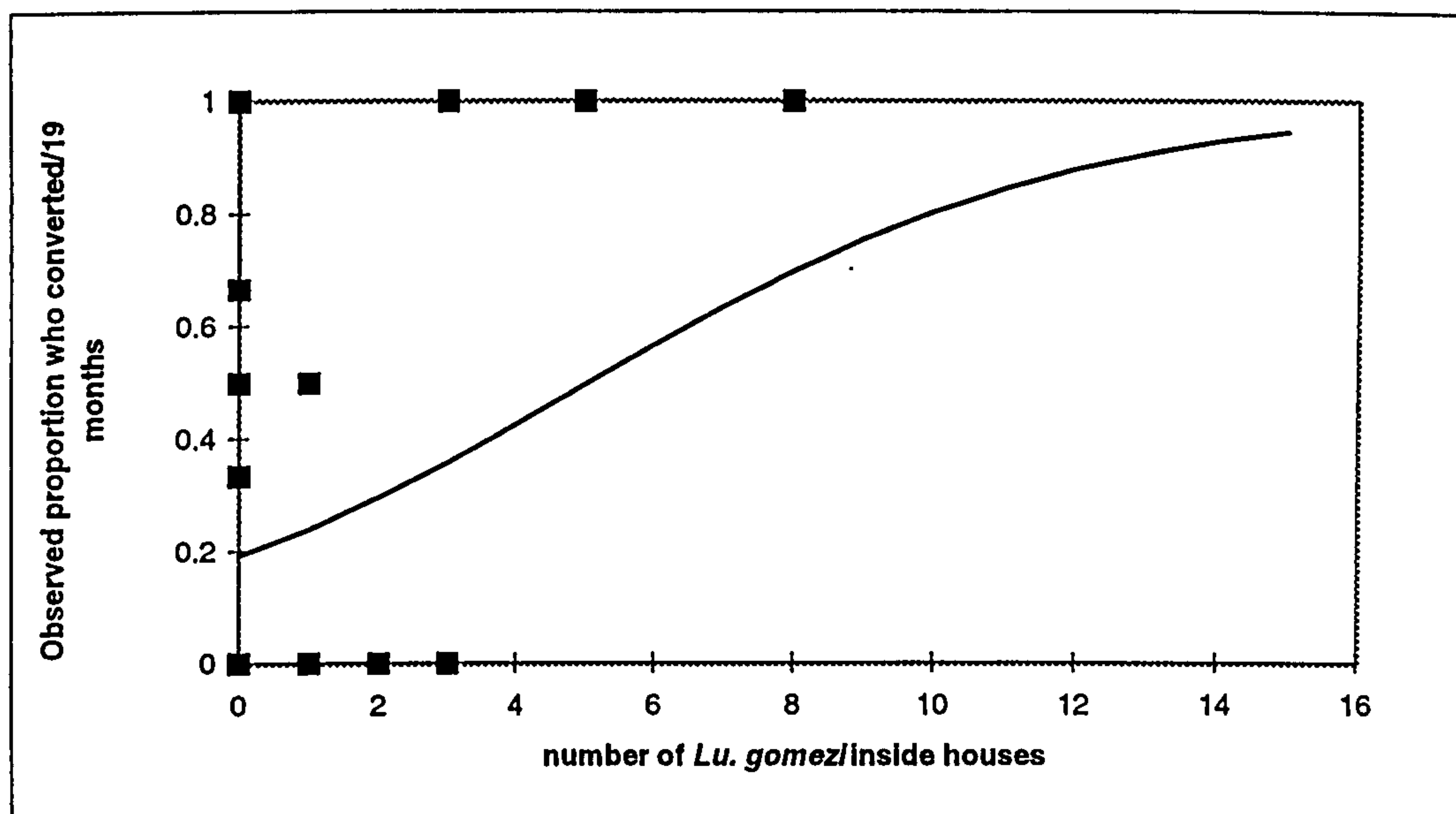
In the analysis of incidence rate, a minimal adequate model with only one explanatory variable was obtained by backward elimination from a

maximal model with seven entomological variables. The indoor abundance of *Lu. gomezi* was positively correlated with the household incidence rates of infection ($\chi^2 = 6.26$; $P < 0.01$; 1 D.F.). The model predicts that an increase in 1 *Lu. gomezi* female / 10 house-nights causes an increase of 4% ($e^{0.1 \times 0.43}$) in the odds of getting infected (Table 5.11). However, the strength of the association was relatively low: $r^2 = 0.08$ i.e. only 8% of the variance in household incidence rate was explained by variation in *Lu. gomezi* indoor abundance (Figure 5.7).

Table 5.11 Sandfly activity inside houses vs incidence and prevalence
Parameter estimates, standard errors and r^2 for the minimal adequate
models generated by multiple regression analyses

	variable	estimate	(S.E)	
SANDFLY vs INCIDENCE (HOUSE)				
INTERCEPT		-1.438	(0.284)	*
SLOPE	<i>gomezi</i>	0.435	(0.205)	
r^2		0.080		
SANDFLY vs PREVALENCE (HOUSE)				
INTERCEPT		0.936	(0.153)	**
SLOPE	<i>trapidoi</i>	0.184	(0.078)	
r^2		0.050		
SANDFLY vs INCIDENCE (VILLAGE)				
INTERCEPT		-1.601	(0.285)	*
SLOPE 1	<i>gomezi</i>	1.069	(0.560)	
SLOPE 2	<i>trapidoi</i>	0.531	(0.389)	
r^2		0.380		
SANDFLY vs PREVALENCE (VILLAGE)				
INTERCEPT		0.736	(0.098)	***
SLOPE	<i>trapidoi</i>	0.828	(0.157)	
r^2		0.690		

Figure 5.7 The relationship between indoor abundance of *Lu. gomezi* and household incidence rate



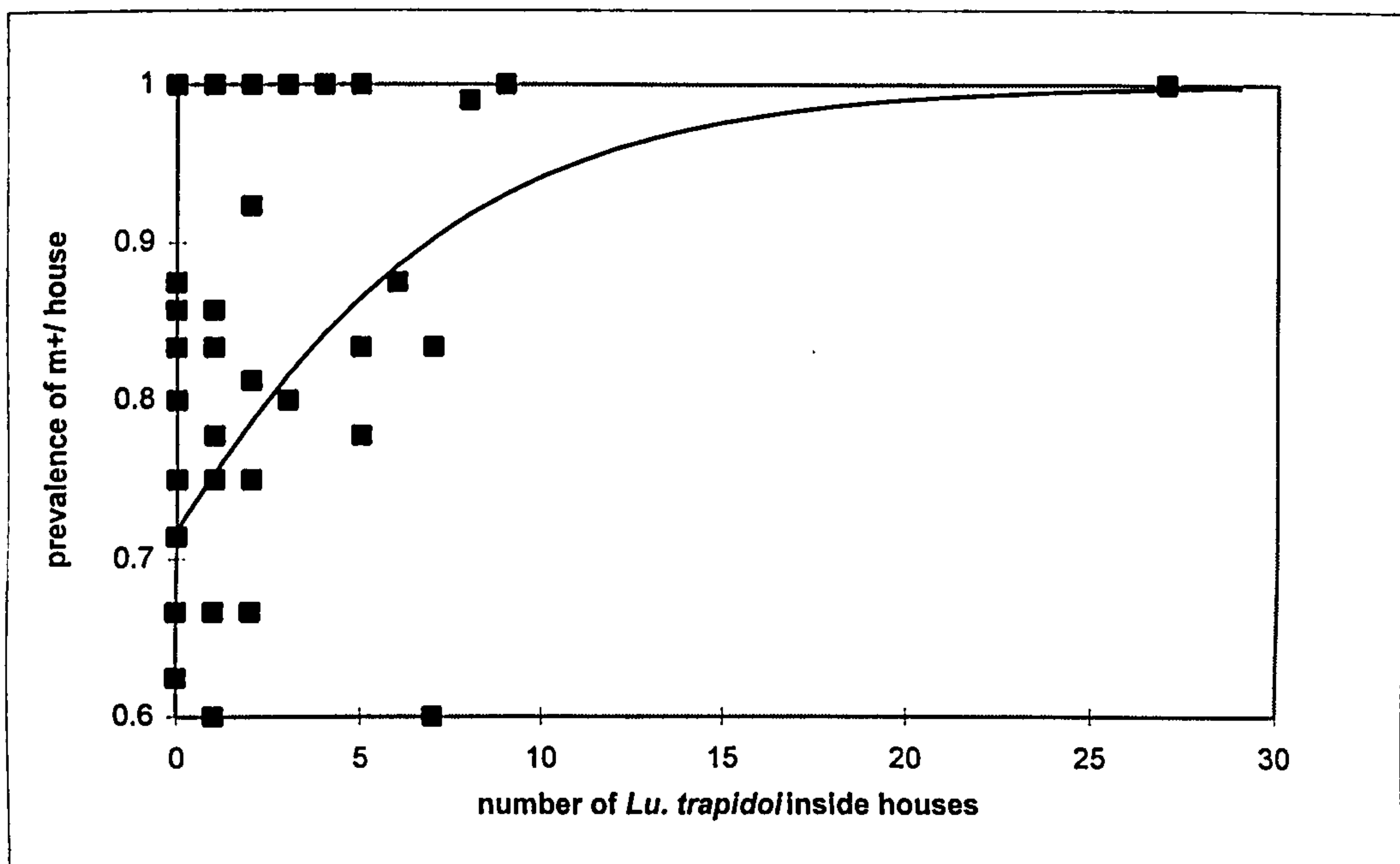
The squares are observed data; the line was fitted by regression analysis (assuming binomial errors)

b) Prevalence

In the analysis of prevalence, a minimal adequate model with only one explanatory variable was obtained as in the analysis of incidence rate. However, in this case, the abundance of *Lu. trapidoi* was positively correlated with the household prevalence of infection ($\chi^2 = 9$; $P < 0.005$; 1 D.F.). The model predicts that an increase in 1 *Lu. trapidoi* female / 10 house-nights is associated with an increase of 1.8% ($e^{0.1 \times 0.184}$) in the odds of being infected (Table 5.11). The strength of the association, as for the household incidence rate model, was relatively low: $r^2 = 0.05$ i.e. only 5% of the variance in

household prevalence was explained by variation in the geometric mean of *Lu. trapidoi* indoor abundance (Figure 5.8).

Figure 5.8 The relationship between indoor abundance of *Lu. trapidoi* and household prevalence (m+)



The squares are observed data; the line was fitted by regression analysis (assuming binomial errors)

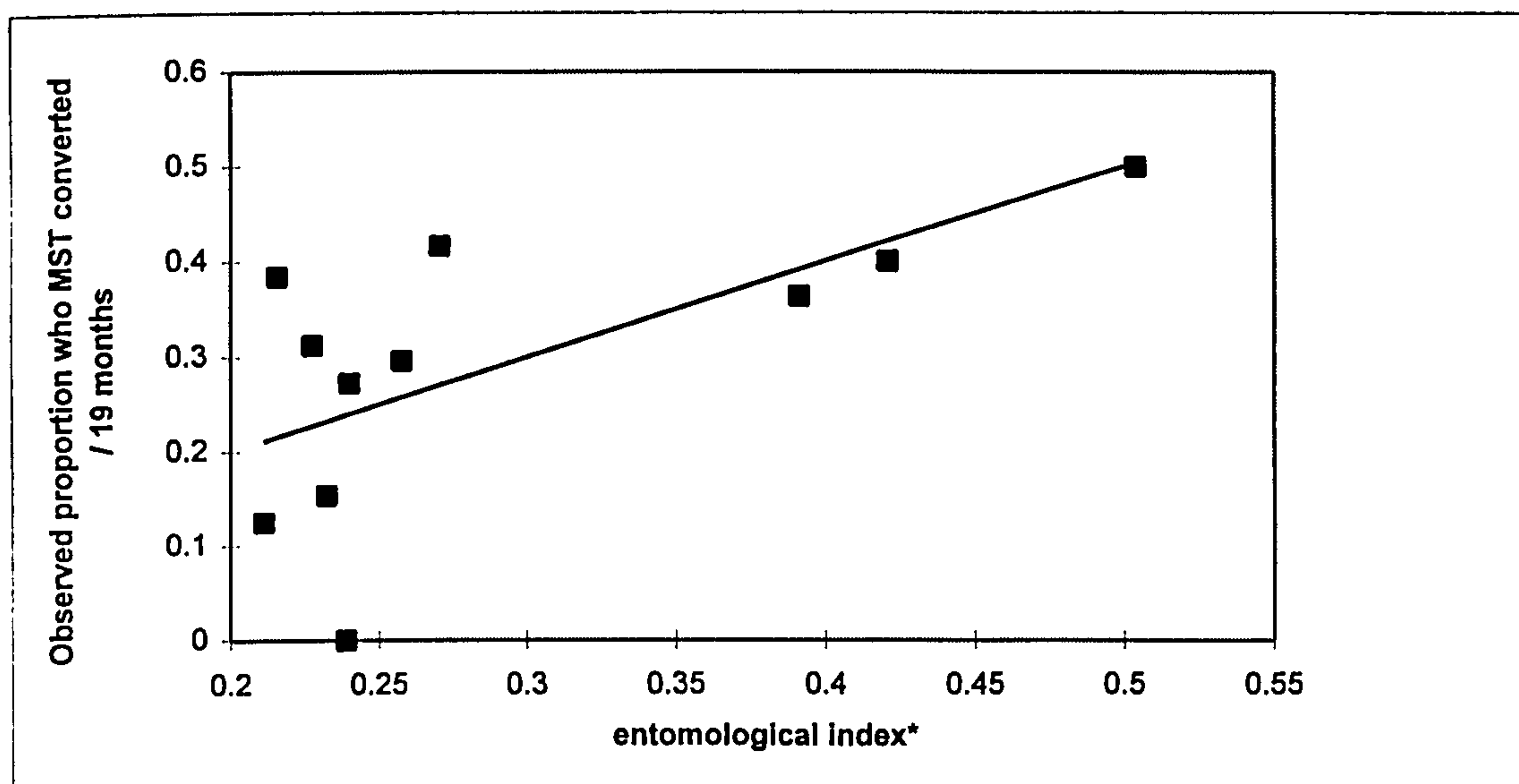
5.2.5.2 Variation at the village level

a) Incidence

In the analyses of village incidence rate, the geometric mean abundance of each sandfly species collected in each village were the explanatory variables, and the outcome variable was the proportion of people

in each village who converted to M+ during the 19 months cohort study. In the MAM the abundance of both *Lu. gomezi* ($\chi^2 = 4.23$; $P < 0.05$; D.F 1) and *Lu. trapidoi* ($\chi^2 = 3.96$; $P < 0.05$; D.F 1) were positively correlated with the incidence rate of infection per village. The model predicts that an increase in one female *Lu. gomezi* or one female *Lu. trapidoi* /10 house-nights, causes an increase of 11% and 5%, respectively, in the odds of getting infected (Table 5.11). The strength of the association was considerably higher than those calculated for household incidence rate: $r^2 = 0.38$ i.e. 38% of the variance in the village incidence rate was explained by variation in the geometric mean of the indoor abundance of both *Lu. gomezi* and *Lu. trapidoi* (Figure 5.9).

Figure 5.9 The relationship between an entomological index, incorporating the mean village abundance of both *Lu. trapidoi* and *Lu. gomezi* and the village incidence rate

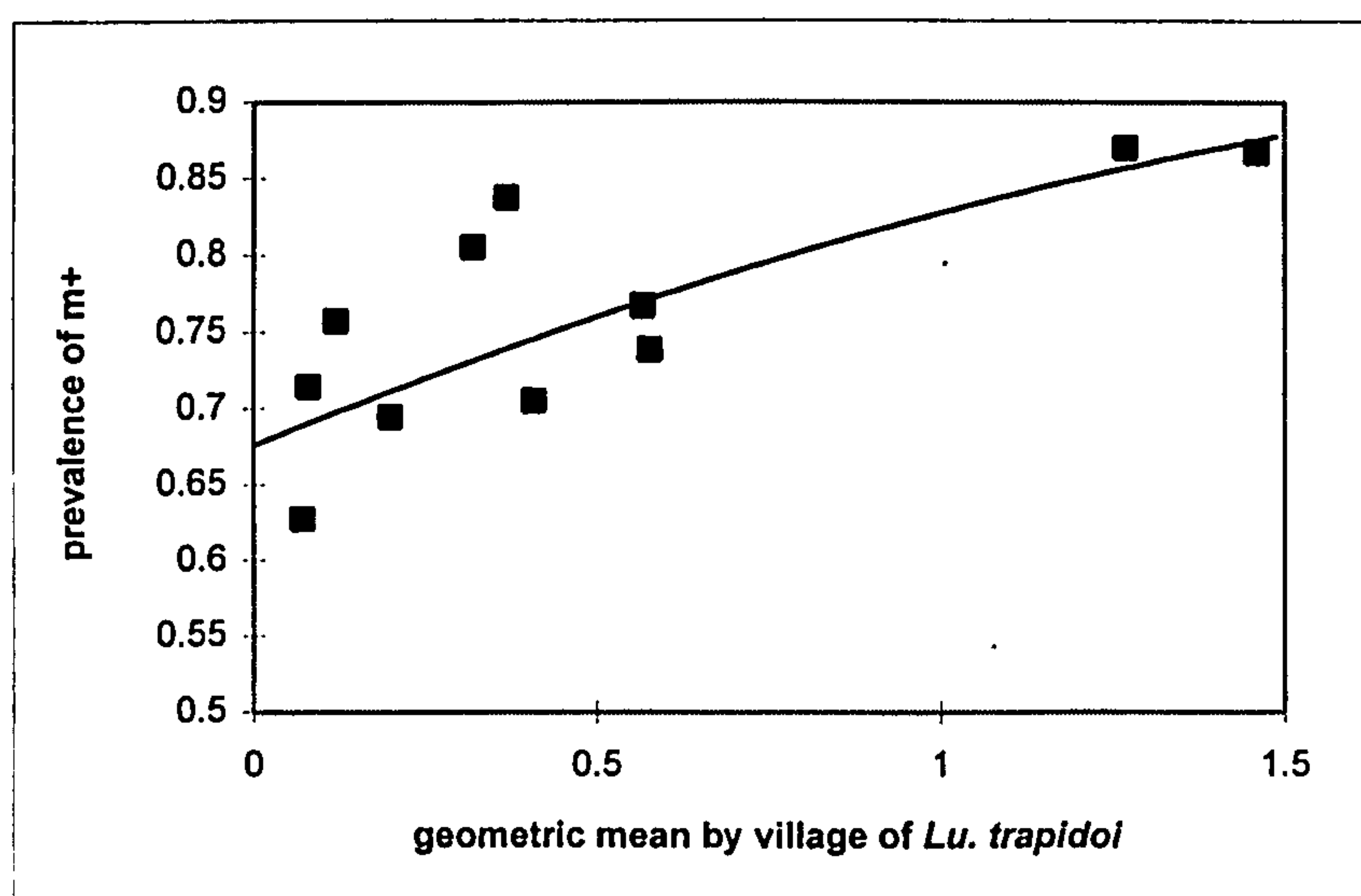


*Entomological index incorporating the mean number of both, *Lu. trapidoi* and *Lu. gomezi*. Squares represent observed village incidence. Line was fitted by regression analysis

b) Prevalence

As for the village incidence rates, the analysis of village prevalence used the geometric means of sandfly abundances as the explanatory variables, but in this case the outcome variable was the proportion of each village population with a positive MST response at the first sectional survey. In the MAM, the abundance of *Lu. trapidoi* was positively correlated with the prevalence of infection per village ($\chi^2 = 30.83$; $P < 0.0001$; 1 D.F.); the model predicts that an increase in 1 of female *Lu. trapidoi* /10 house/nights causes an increase of 8.6% in the odds of being infected (Table 5.11). The association between *Lu. trapidoi* abundance and village prevalence rate was remarkably high: $r^2 = 0.69$, i.e. 69% of the variance in the village prevalence was explained by the variation in the geometric mean of *Lu. trapidoi* indoor abundance (Figure 5.10).

Figure 5.10 The relationship between the mean village abundance of *Lu. trapidoi* and village prevalence (m+)



The squares are observed data; the line was fitted by regression analysis (assuming binomial errors)

5.2.6 Seasonal variation

In the seasonal variation study, seven sandfly species were caught between February 1996 and January 1997 at eight sites (18 man-landing collections/site [19:00 to 22:00 hours]), representing four habitat types (two replicates/habitat type), in and around Houses 1 and 2 in San Pedro: (1) indoors, (2) in the peri-domicile, (3) in cacao and (4) in primary forest (Table 5.2). The number of individuals collected of *Lu. gomezi*, *Lu. yuilli*, *Lu. panamensis* and *Lu. erwindonaldi* were too low to draw any conclusions about their seasonal patterns. This section describe the analysis of seasonal variation for the other three species collected: *Lu. hartmanni*, *Lu. trapidoi* and *Lu. quasitowsendi*. The results should be viewed with caution because: (1) the total number of sandflies collected for each species was relatively low, (2) there was no temporal correlations between the sandflies collections made at the paired replicates (i.e. sites with similar habitats type), and (3) there was no temporal correlation between the collections made in different habitat types within the same locality.

Nevertheless, there was some evidence for species-specific seasonal patterns in abundance. The abundance of *Lu. hartmanni* decreased significantly from a geometric mean (GM) of 22.1 (95% C.I. 14.6 - 33.4) sandflies/month from February to July to a GM of 11.1 (95% C.I. 6 - 20.3)

sandflies/month from September to January (ANOVA: $F= 4.9$, $p<0.05$) (Figure 5.11). *Lu. quasitowsendi* populations also decreased significantly from a GM of 4.6 (95% C.I. 2.2 - 8.6) sandflies/month from February to July to a GM of 1.3 (95% C.I. 0.7 - 3) sandflies/month from September to January (ANOVA: $F= 5.5$, $P <0.05$) (Figure 5.12). In contrast, the *Lu. trapidoi* population was relatively stable throughout the year with no significant seasonal trend (Figure 5.13).

No meteorological correlates of the seasonal patterns of sandfly abundance were identified, in part because most climatic variables in Opon are remarkably constant throughout the year. For example, minimum daily temperature was very stable throughout the year, with a minor peak in April and May, when the monthly mean ranged from 20.8°C to 20.9 °C, compared to a range of 18.9°C - 20°C during the rest of the year (Figure 2.10 and Table 2.1). The monthly mean maximum temperature ranged from 31.6°C to 35.2°C during the year, with slight peaks in April and June (Figure 2.11 and Table 2.1). Relative humidity (RH) showed only minor variation through the year, with monthly means ranging from 88.5% to 93.6% in all months, except July which peaked at 97.9% (Figure 2.10 and Table 2.1).

In the Opon focus there is no dry season, i.e. there is no month with no rain, but the total monthly rainfall clearly peaks twice during the year from March to June (ranging from 207 - 369 mm.) and from October to November

(from 274 - 275 mm) (Figure 2.8 and Table 2.1). During the rest of the year, the monthly mean total rainfall ranged from 51 to 150 mm. However, when the frequency of rainfall (i.e. the proportion of days with some rainfall) was plotted, the bimodal pattern disappeared. Rainfall frequency had a single peak in April and dropped gradually to a minimum in December (Figure 2.9 and Table 2.1).

For all three species analysed, no relationship was detected between sandfly abundance and any of the five meteorological measurements tested (minimum and maximum temperature, relative humidity, total rainfall, and frequency of rainfall) either during the same month as the sandfly collection or in any of the three previous months. However, the gradual reduction in abundance of *Lu. quasitowsendi* from May to December was most closely mirrored by the seasonal pattern in the frequency of rainy days. In a linear regression model, rainfall frequency during the previous 30 days explained 20% of the variance in *Lu. quasitowsendi* abundance (Figure 5.14), but the F value was just outside the borderline ($F = 4.4$) for significance : $F = 4.0$; D.F = 1,16; $P > 0.05$. Of course, given the large number of regression analyses carried out, one must be very wary of drawing firm conclusions from a single marginally significant result.

Seasonal changes in the population size of adults sandflies may be influenced by ecological effects acting on any part of the sandfly life cycle. In

contrast, the behavioural decision made by sandflies whether or not to enter houses for a blood meal (i.e. endophagy) will be determined by conditions, such as the weather, on the same night. Hence, explanations for the seasonal patterns in endophagic behaviour were sought by investigating possible correlations with the weather conditions on each collection night. Endophagy was measured for each species as the proportion of the total night's catch which was from the indoor man-landing collection. This proportion apparently peaked twice during the season for all three species: for *Lu. hartmanni* and *Lu. trapidoi* in July and October, and for *Lu. quasitowsendi* in June and from November to December (Figure 5.15). However, no significant correlations were detected between endophagic activity for any of the three species tested and the weather conditions on the sampling night.

Figure 5.11 Seasonal variation of *Lu. hartmanni*

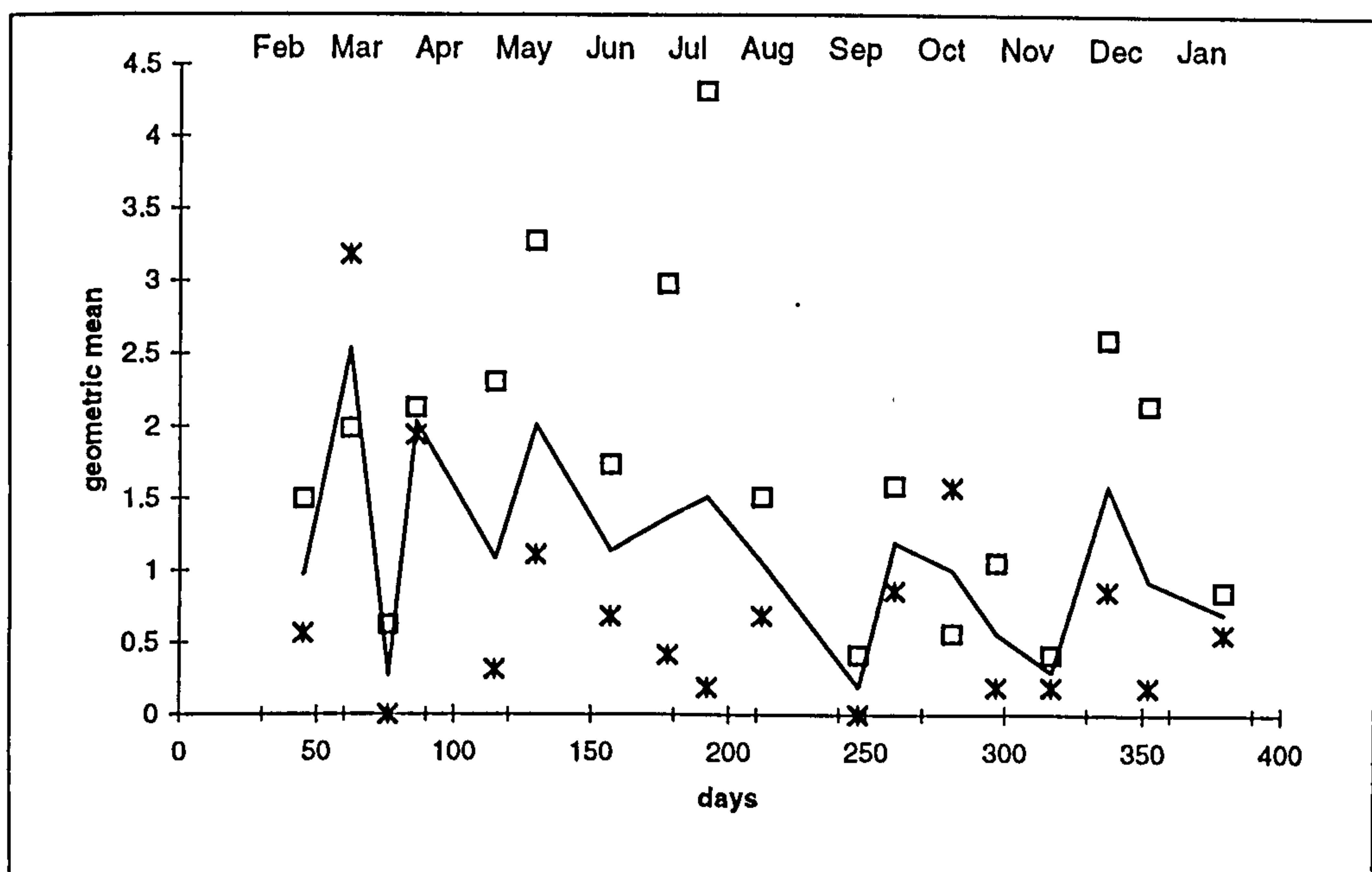


Figure 5.12 Seasonal variation of *Lu. quasitowsendi*

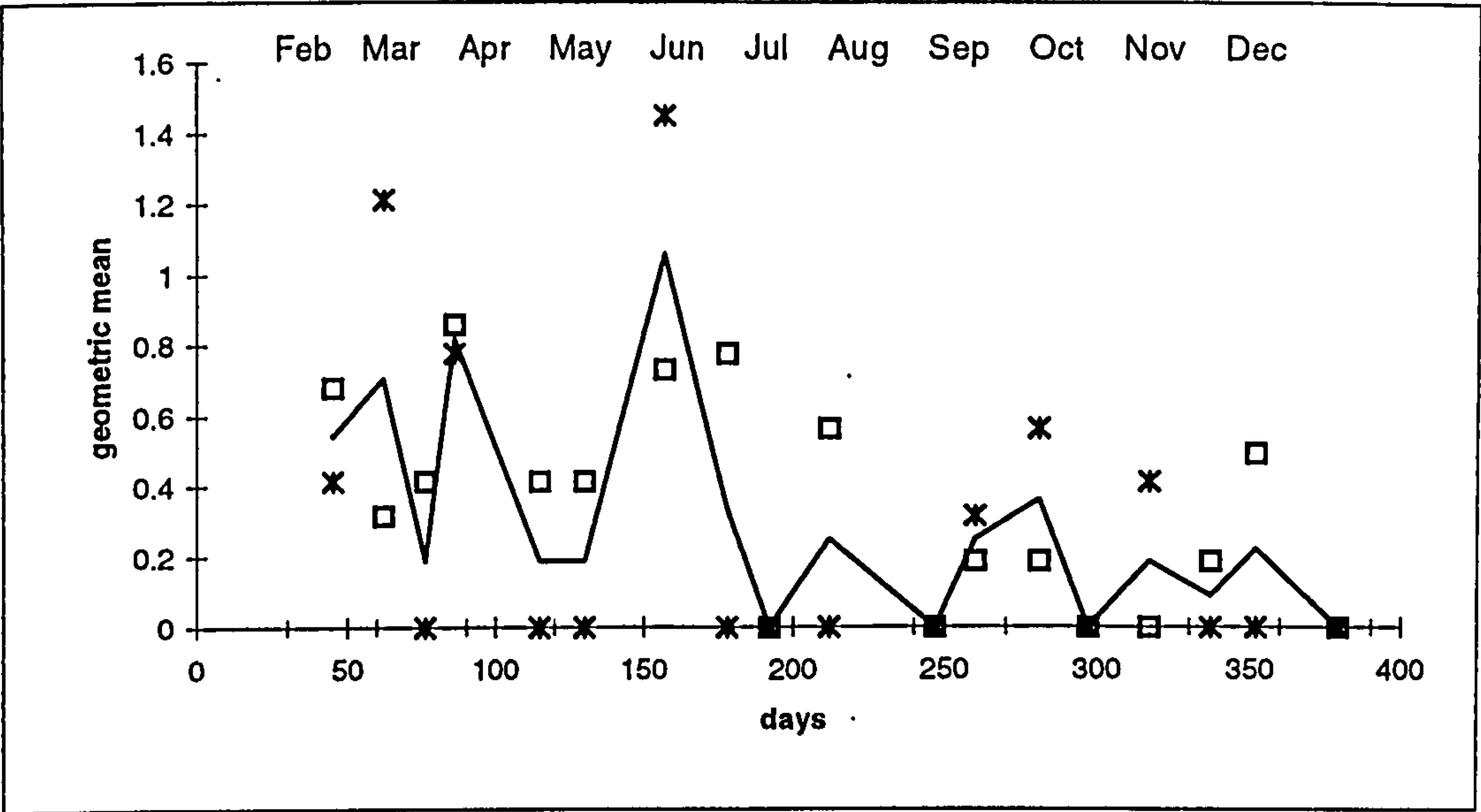
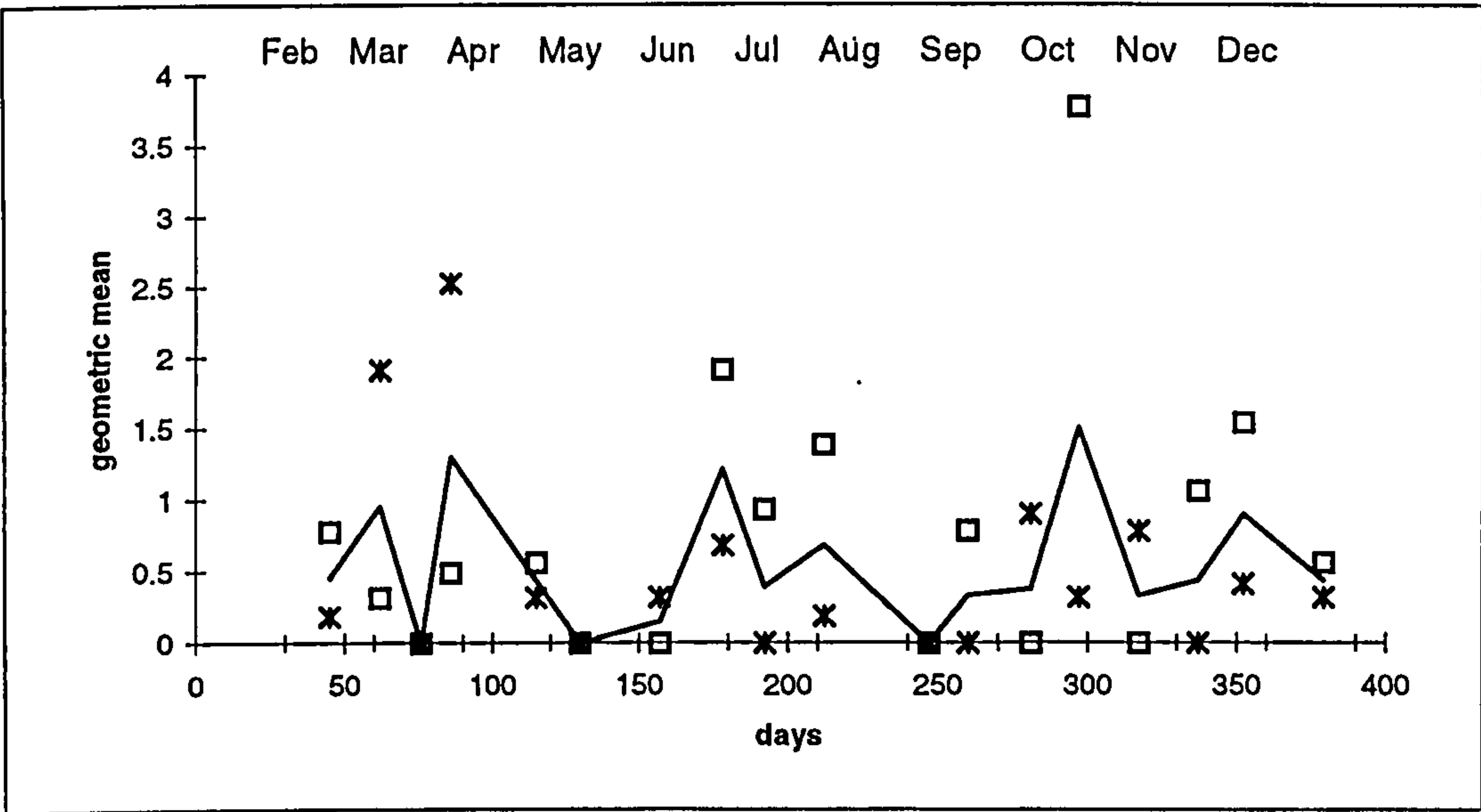
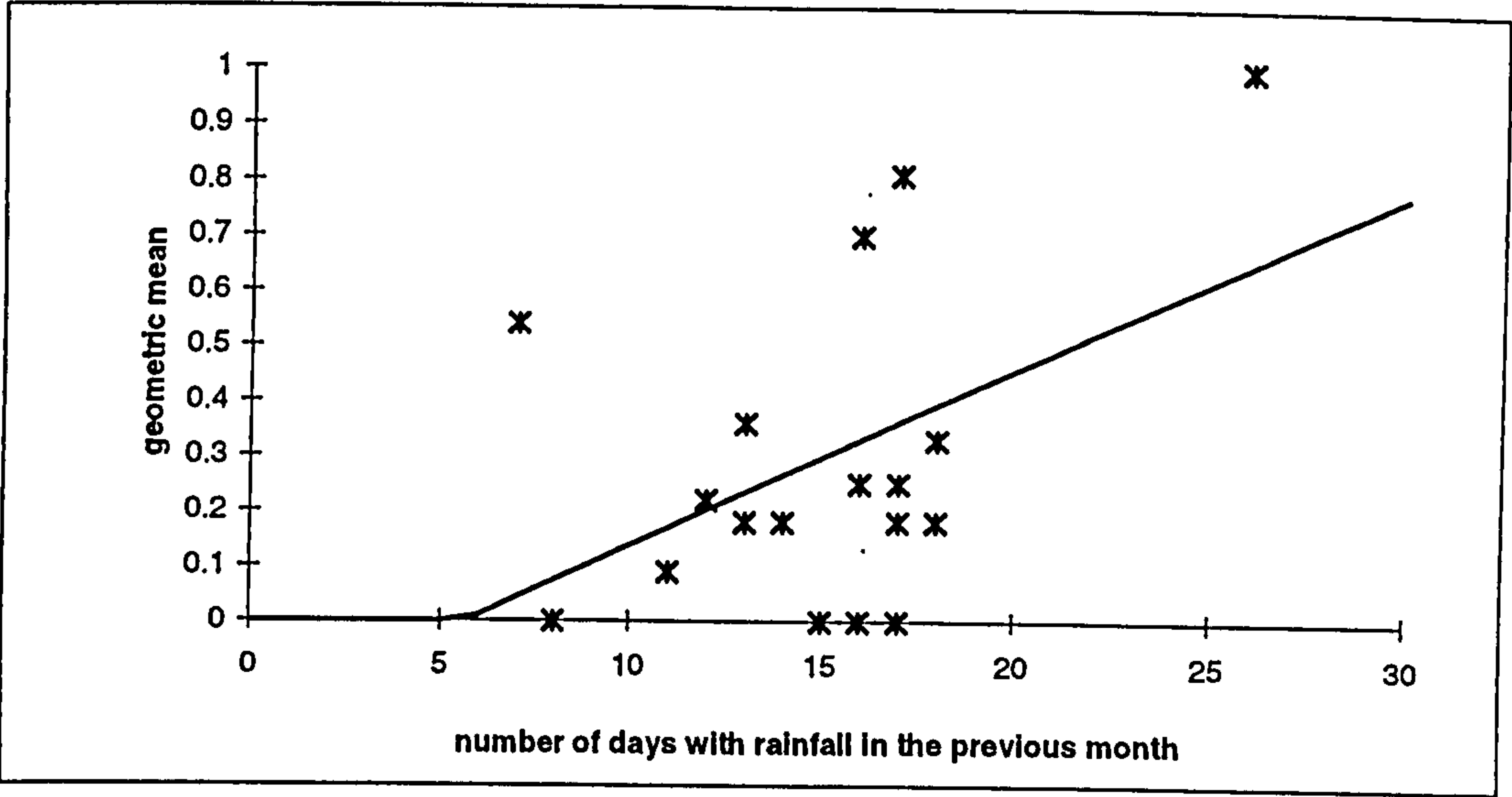


Figure 5.13 Seasonal variation of *Lu. trapidoi*



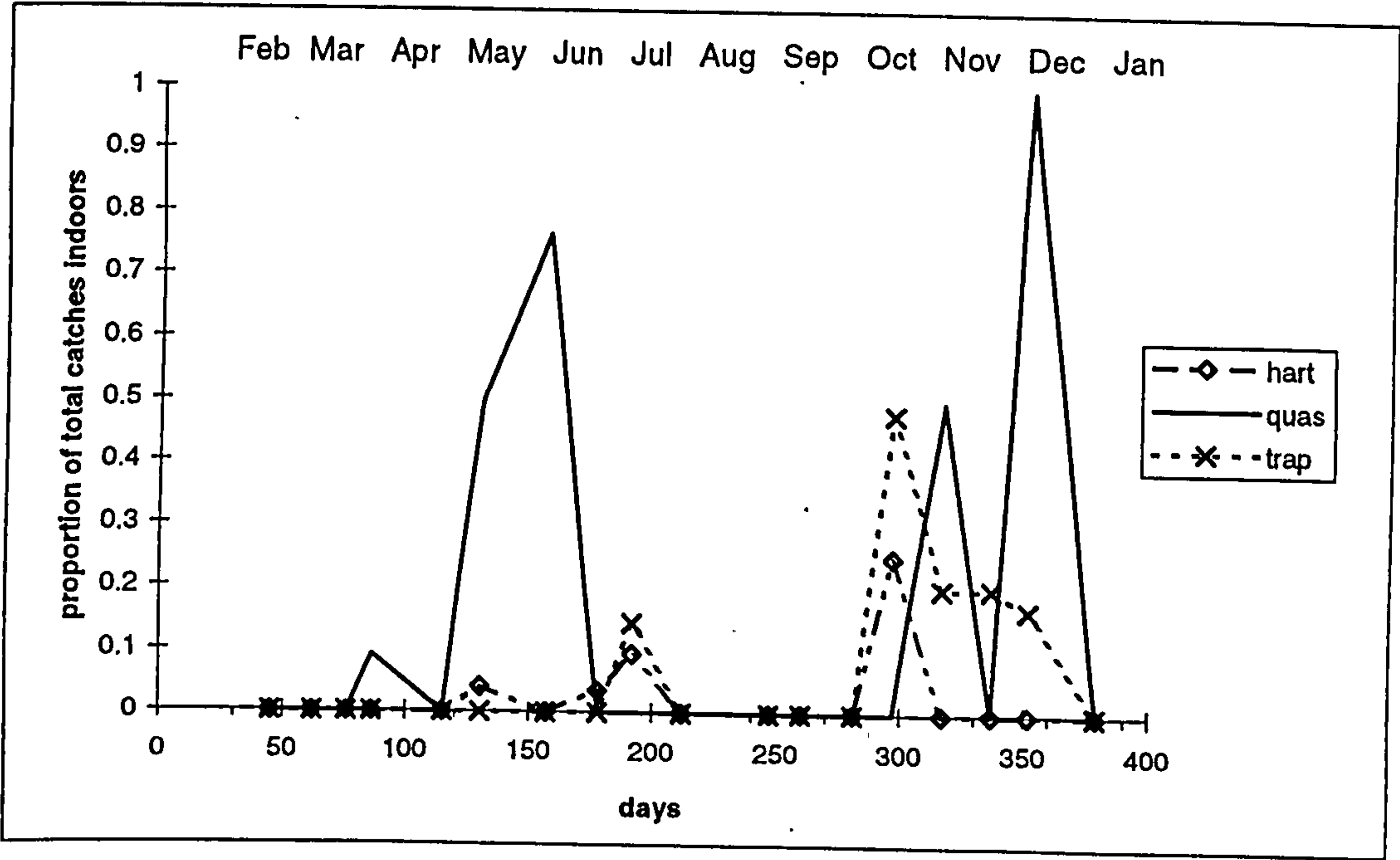
Legend for Figures 5.11 to 5.13: The Lines shows the geometric mean of the number of sandflies collected in eight sites, representing four habitat types (in and around Houses 1 and 2). The squares are the geometric means for sandflies in and around House 1, and the asterisks are the geometric means for sandflies in and around House 2 (day 0 = 1 January 1996)

Figure 5.14 Relationship between rainfall frequency and the subsequent abundance of *Lu. quasitowsendi*



Asterisks represent the observed values. The line was fitted by linear regression.

Figure 5.15 Seasonal variation in endophagic activity of sandflies in the Opon focus



5.3 DISCUSSION

In Colombia, the most comprehensively reported previous studies of the behaviour and ecology of putative sandfly vectors of *L. panamensis* were carried out in Tumaco (Travi et al, 1988), Antioquia (Velez et al, 1992; Porter & DeFoliart, 1981) and Valle de Cauca (Alexander et al, 1995a,b). However, in the Department of Santander, in general, and in the Opon focus in particular, there are no previously published quantitative data on either sandfly behaviour or ecology. Indeed, the only previously published sandfly study in this region appears to be a single day sandfly collection, where the sole aim was to provide information on the geographic distribution of sandfly species (Osorno-Mesa et al, 1972). Thus, it is hoped that the results and discussion presented in this chapter should contribute significantly to the general comprehension of the biology of the sandfly vectors of *L. panamensis* in Colombia.

5.3.1 Vectors of *Leishmania panamensis*

In the Opon focus, the diversity of sandfly fauna (27 species were identified) is relatively high compared with the results in other *L. panamensis* foci: for example, 13 spp in Guatemala (Rowton et al, 1991); 11, 17 and 26 in Colombia spp (Travi et al 1988, Lopez et al, 1996 ; Barreto et al, 1989

respectively); 25 spp in Ecuador (Le Pont et al, 1994a) and 26 spp in Panama (Chaniotis 1971a,b). However, the reported species diversity and relative abundance varies according to the collection methods employed.

In the Opon focus, five anthropophilic species comprised 87% of the total sandfly collection (independent of the trap method): *Lu. trapidoi*, *Lu. hartmanni*, *Lu. quasitowsendi*, *Lu. gomezi* and *Lu. ovallesi*. Amongst these species, only *Lu. trapidoi* and *Lu. gomezi* are currently proven vectors of *L. panamensis* (Table 5.12). *Lu. hartmanni* and *Lu. ovallesi* are currently suspected vectors because they have been found with unidentified flagellates in *L. panamensis* foci (Hashiguchi et al, 1985a). At least five other species have also been described as suspected vectors of *L. panamensis*: *Lu. panamensis*, *Lu. shannoni*, *Lu. edentula*, *Lu. ylephiletor* and *Lu. sanguinaria* (Killick-Kendrick, 1990). The last three species are important only in Central America (Young, 1987a; Killick-Kendrick, 1990). *Lu. panamensis* and *Lu. shannoni* were collected in the Opon focus, but are unlikely to have a significant vectorial role there because: (1) *Lu. panamensis* is rare - its relative abundance was 0.8% of the whole sandfly collection (Table 5.1); and (2) the anthropophilic activity of *Lu. shannoni* was very low - 3% of the *Lu. shannoni* collected were caught on human bait (Table 5.1).

5.3.2 Natural infection of sandflies

Natural infections with flagellates were found in 1.2% of the dissected sandflies, but a relatively low proportion of the flagellates were typed as *Leishmania* parasites belonging to the *L. braziliensis* complex (2/21. i.e. 10%). In previously published studies of suspected vectors of *L. panamensis*, the proportion of females naturally infected with flagellates ranged from 0 - 13.5% (Table 5.12). As in the Opon study, these percentages are significantly higher than those recorded for females found with typed *Leishmania* parasites (range: 0 - 2%). The usual explanation for this discrepancy is the logistic difficulties in the *in vitro* isolation of parasites from sandflies: the culture medium is frequently contaminated with bacteria, and isolation is often unsuccessful for slow growing parasites. However, the present studies in the Opon focus, *in vitro* isolation attempts were avoided, being replaced with PCR and inoculation in hamsters. Thus, the most likely explanation for the relatively high frequency of flagellates detected in wild caught sandflies is the presence of other trypanosomatids, morphologically indistinguishable from the *Leishmania* spp. responsible for human disease. Indeed, Christensen et al (1983) in Panama found that the intra-erythrocytic flagellate *Endotrypanum schaudinni* establishes heavy infections in both *Lu. gomezi* and *Lu. trapidoi*. The reliability of PCR for the detection of natural infections has been proven in *L. braziliensis* foci; for example in a Venezuelan study (Felicangeli et al, 1994), a total of 4,863 sandflies were dissected, and

53 (1.09%) harboured promastigotes in the gut. Microscopic examination was used as the "gold standard", and PCR was carried out on all positive guts. All parasites detected in microscopic examination were also detected and identified (*L. braziliensis*) by PCR. In spite of the perfect correlation between observed vs. classified parasites, the infection rate was still very low.

Although the natural infection of *Lu. quasitowsendi* with flagellates in the Opon area is the first registered record for this species, the significance of this finding will only be clarified when the flagellates are characterised. *Lu. quasitowsendi* belongs to the verrucarum series of the *Lu. verrucarum* group, in which at least three species are already suspected or confirmed vectors of leishmaniasis: *Lu. spinicrassa*, *Lu. youngi* and *Lu. townsendi* (Young et al, 1987c; Warburg et al, 1991; Rowton et al, 1991). Hence, *Lu. quasitowsendi* should be considered a target for future work on vector incrimination.

The accumulated incriminatory evidence from natural infections is clearly more convincing for *Lu. trapidoi* than for *Lu. gomezi* (Table 5.12). At least 11 characterised infections have reportedly been detected in field caught *Lu. trapidoi* (from 5 studies) compared to only one in *Lu. gomezi*. The other biological evidence available also generally supports the contention that *Lu. trapidoi* is a more efficient vector than *L. gomezi*: (1) *Lu. trapidoi* feeds on a broad range of mammals, indicating that host selection is influenced by

availability, but bloodmeals taken from sloths (*Choloepus hoffmanni*), the proven reservoir of *Leishmania panamensis*, have been found in wild *Lu. trapidoi* individuals (Tesh et al, 1971, 1972; Zeledon et al, 1985; Christensen et al, 1983), and this species has also been observed biting sloths (Thatcher & Herting, 1966); whereas, *Lu. gomezi* feed mainly on primates (Zeledon et al, 1985). (2) Experimental infections conducted by Jaramillo et al (1994) on both sandfly species, using hamsters infected with *L. panamensis*, showed that *Lu. trapidoi* developed promastigote infective forms by day 5 post infection, whereas *Lu. gomezi* at the same day harboured only paramastogotes adhering to the pylorus.

Another criteria useful for vector incrimination is the identification of a significant overlap between the geographic distribution of sandflies and the distribution of parasites. However, as mentioned above, the records of sandfly fauna and the relative abundance of sandfly species in each foci could be biased by the trapping methodology used. In the following section, the relative efficiency of the two main methods used in the Opon focus for sandfly collections are discussed.

Table 5.12 Natural infection rates in Colombian sandflies which are proven or suspected vectors of *L. panamensis*

sandfly species	# dissected	# with flagellate	%	# typed as <i>L. pan.</i>	%	Reference
<i>Lu. trapidoi</i>	2,869	42	1.50	3	0.10	Morales et al, 1981
	2,789	375	13.5	3	0.11	Christensen et al, 1983
	491	38	8.1	ns	ns	Hashiguchi et al, 1985a
	51	6	11.8	1	2	Zeledon et al, 1985
	2,734	10	0.36	2	0.10	Travi et al, 1988
	926	0	0	0	0	Loyola, et al, 1988
	6,965	454	6.5	ns	ns	Le Pont et al, 1994b
	558	11	2	2	0.35	Opon focus, 1996
<i>Lu. gomezi</i>	383	1	0.26	0	0	Morales et al, 1981
	940	40	4.30	1	0.11	Christensen et al, 1983
	1,410	3	0.21	0	0	Travi et al, 1988
	165	3	1.8	0	0	Opon focus, 1996
<i>Lu. panamens.</i>	1,274	18	1.40	1	0.08	Christensen et al, 1983
	434	1	0.23	0	0	Travi et al, 1988
	20	0	0	0	0	Opon focus, 1996
<i>Lu. hartmanni</i>	409	0	0	0	0	Morales et al, 1981
	563	22	3.90	ns	ns	Hashiguchi et al, 1985a
	665	0	0	0	0	Travi et al, 1988
	106	0	0	0	0	Opon focus, 1996

5.3.3 Collection methods

Man-landing collections were used in Opon for identifying sandfly temporal characteristics and for the assessment of natural infection rates, on the basis that this method is the most direct entomological measure of transmission rate to humans. However, for logistic reasons, CDC light traps were chosen for the comparative study of endophagic activity. Interpretation of the light trap data needs to take into account the potential bias that this method could generate in providing an estimate of sandfly abundance and species composition inside houses. Sandfly species will differ both in their relative attractiveness to light and in their relative attractiveness to human hosts (Travi et al, 1988); and, of course, human bait catches will be heavily female-biased, whereas both genders are likely to be attracted to light (Davies et al, 1995b), though not necessarily to an equal degree (Gibb et al, 1988).

In Opon, the light trap data clearly overestimates the relative risk of being bitten by *Lu. gomezi* in comparison with the risk of being bitten by *Lu. trapidoi*. This is because *Lu. gomezi* is more phototropic and/or because *Lu. trapidoi* is more anthropophilic. The light trap bias for indoor *Lu. gomezi* is unlikely to be due to an influx of *Lu. gomezi* attracted indoors by the light source, because the distance from which phlebotomine sandflies are

attracted to light is not thought to exceed 6 m (Odetoyinbo, 1969; Valenta et al, 1995).

With some exceptions (Davies et al, 1995b), previous studies of the relationship between light trap and human bait catches of Neotropical sandflies are largely anecdotal. However, for *L. panamensis* vectors, there are at least three published studies which report the results of sandfly captures by both human bait and light trap: Morales et al (1981) and Travi et al (1988) in Colombia and Le Pont et al (1994a) in Paraiso Escondido, Ecuador (Table 5.13). In all cases, the ratio of *Lu. gomezi* : *Lu. trapidoi* was higher in the light trap collection than in the human bait catch. A stratified chi square analysis of all three results shows that this ratio is on average 53% greater in the light trap than in the human bait catches ($\chi^2 = 73.23$; $P < 0.001$; $RR = 1.53$; Greenland / Robins Confidence Limits, 1.39 - 1.68). When the Opon focus results are added to the stratified analysis, the Relative Risk increases to 1.56 ($\chi^2 = 81.45$; $P < 0.001$; Greenland / Robins Confidence Limits, 1.51 - 1.79).

Table 5.13 Relationship between light trap and human catches of sandflies in four studies in Andean Countries

author	man-landing collections (<i>Lutzomyia</i> spp.)				CDC light traps (<i>Lutzomyia</i> spp.)			
	<i>trapidoi</i>	%	<i>gomezi</i>	%	<i>trapidoi</i>	%	<i>gomezi</i>	%
Morales	3,681	88	487	12	324	80	61	20
Travi	2,289	63	1340	37	16	19	70	81
LePont	21	26	60	74	72	10	1277	90
Opon/focus	23	88	3	12	13	48	14	52

5.3.4 Vector incrimination by regression analysis

In the Opon focus there is strong epidemiological evidence that transmission of leishmaniasis to humans is largely domestic because: (1) the risk of infection was unrelated to gender or age, and (2) children are more likely than adults to have lesions or scars on their head (presumably as their head is exposed when bitten in bed) (see Chapter 3). The entomological data provide further support for the potential for domestic transmission. In particular, (1) suspected vectors were collected both indoors and in the peridomestic environment, (2) the sex ratio of the anthropophilic species caught indoors (by light trap) was highly female biased (Table 5.1), indicating that the principal activity in the domestic habitat is blood-feeding activity, (3) the risk of infection for a member of a particular household was shown to be statistically correlated with the abundance of the suspected vectors inside their house. The majority of houses in Opon do not represent a physical barrier for sandflies because they are made of wood, with wide spaces between planks and with an open space between roof and walls, i.e. the houses have similar characteristics to those in a Costa Rica study, where a 1:1 relationship was obtained between *Lu. gomezi* collected inside and outside houses (Herrero et al, 1992) .

Amongst the several anthropophilic species collected inside houses in the Opon focus, only *Lu. trapidoi* and *Lu. gomezi* abundance were

significantly associated with transmission rate (incidence or prevalence). Hence, the conclusions drawn from the regression analyses are consistent with the conclusions drawn from previous studies of *L. panamensis* transmission, as both *Lu. gomezi* and *Lu. trapidoi* have been incriminated as vectors previously (see above). This appears to be only the second reported use of multiple regression analysis to evaluate the vectorial roles of New World sandflies. Previously, Davies et al (1997b) found that the transmission rate of *L. peruviana* was significantly associated with the abundance of those sandflies species for which there was some independent biological incriminatory evidence.

The parameter values which measure the relationship (i.e. the regression slope) between indoor sandfly abundance and leishmaniasis transmission rates (in the minimal adequate models) describe the relative roles of the two most likely vectors in the Opon focus. Indoor activity of *Lu. trapidoi* was related both with the village incidence rate of leishmaniasis at the time when this study was conducted, and also with the average transmission rate in previous years (which is crudely summarised by the cumulative prevalence of infection). By contrast, *Lu. gomezi* indoor activity was related only with incidence rate. This may be because *Lu. gomezi* has only recently developed a significant vectorial role in the Opon focus, possibly as a result of the change in habitat caused by the rapid deforestation in that area. Not surprisingly, sandfly abundance was a better predictor of village transmission

rate ($r^2 = 69\%$ for prevalence and 38% for incidence) than of household transmission rates ($r^2 = 5\%$ for prevalence and 8% for incidence). The reasons for this differential include, (1) the village regressions involved mean sandfly abundance measurements, whereas the household regression involved a single sandfly capture per house, and (2) the transmission rate measurements for villages are based on much larger population sizes than the transmission rate measurements for households. The association between sandfly abundance and household cumulative prevalence rates is also likely to be especially weak, as the cumulative prevalence rate will be highly dependent on the age structure of a household (which is very variable, unlike the age structure of a village). Although the strongest association was between *Lu. trapidoi* abundance and village prevalence, both *Lu. trapidoi* and *Lu. gomezi* were retained in the MAM with incidence as the outcome variable; and in this MAM the slope for *Lu. gomezi* (1.069) was greater than that for *Lu. trapidoi* (0.531), suggesting that *Lu. gomezi* is at least as effective a vector as is *Lu. trapidoi* (Table 5.11).

5.3.5 Geographic distribution

Given the epidemiological patterns which are strongly suggestive of domestic transmission (see Chapter 4), the results of the entomological studies indicate that the most important vectors in the Opon focus are *Lu.*

trapidoi and *Lu. gomezi*. The evidence can be summarised as follows: (1) natural infections with flagellates were found for both species, and *Lu. trapidoi* was found with *L. braziliensis* complex parasites (see section 5.2.2); (2) both are distributed in all villages (Table 5.8), (3) *Lu. trapidoi* and *Lu. gomezi* were the two most abundant anthropophilic species collected indoors (Table 5.1); and (4) these were the only two species to demonstrate any significant spatial correlation with transmission rate. However, we cannot discount a minor vectorial role for a third anthropophilic species, *Lu. quasitowsendi*, which was also found with flagellates and was common indoors. Thus, the following discussion of the Opon results and the results from other studies of *L. panamensis* transmission in Central America and Andean Countries focuses on these three species.

The distribution of *L. panamensis* in Central America and Andean Countries overlaps closely with the distribution of *Lu. trapidoi*: (1) *L. panamensis* is not endemic in Guatemala or El Salvador (Grimaldi et al, 1989; Carreira et al, 1995), in which countries *Lu. trapidoi* is rare or absent, respectively (2) *Lu. trapidoi* is abundant in both Costa Rica and Panama, where *L. panamensis* is highly endemic; (3) in areas of Colombia and Ecuador where *L. panamensis* is the most common agent of cutaneous leishmaniasis, *Lu. trapidoi* is the most abundant anthropophilic sandfly species (Belli et al, 1993; Grimaldi et al, 1989; Corredor et al, 1990; WHO, 1990), and (4) neither *L. panamensis* or *Lu. trapidoi* are present in Peru

(Young & Duncan, 1994; Grimaldi et al, 1989). *Lu. gomezi* also overlaps spatially with *L. panamensis*, but this sandfly species is more widely distributed than *Lu. trapidoi*, reaching El Salvador (in the northern part of Central America) and Venezuela, French Guiana, Peru and Trinidad (in South America). Hence, these broad patterns imply a closer association between *L. panamensis* with *L. trapidoi* than with *L. gomezi*.

Lu. quasitowsendi belongs to the *towsendi* series of the *Lu. verrucarum* group. Species in this series are remarkable in their extremely narrow geographic distributions which are usually allopatric. All species in this series are found only in restricted regions of either Colombia or Venezuela. *Lu. quasitowsendi* has only been collected in the Colombian Department of Santander (Young & Duncan, 1994).

5.3.6 Vegetation in relation to sandfly abundance

As described in Chapter II, there are two patterns of deforestation in the Opon focus: (1) partial deforestation, where the majority of trees are cut down but the biggest trees remain in order to provide shade for the cultivation of cacao, and (2) total deforestation, followed by fire and interchange between short-lived crops, like maize or “yuca”, and either pasture or secondary forest. These distinct environmental changes in the tropical rain

forest may have different impacts on sandfly ecology. Hence the effects of these two forms of deforestation in Opon are treated separately below.

a) Cacao crops.

The extent of cacao cultivation around houses was the most consistent positive correlate of intradomiciliary activity for both *Lu. gomezi* and *Lu. trapidoi* (Table 5.9). So there is no evidence that the replacement of primary forest by cacao crops has any major detrimental effect on these sandfly species. Cacao crops can provide sandflies with shade, humidity, breeding places and a sugar source - either directly (e.g. from rotten fruit) or indirectly, by providing an appropriate environment for aphid species, whose honeydew can be a significant nutrient source for New World sandflies (Cameron et al, 1994, and 1995). In addition, sandflies should have access to abundant bloodmeal sources in the cacao crops, due to the rich mammal fauna which can persist, living on the fruits produced by the remaining primary forest trees or by fruit trees (like Zapote tree) planted by farmers. A number of other sandfly vectors have previously been shown to be adapted to cacao plantations; for example, *Lu. whitmani* thrives amongst the cacao of Tres Bracos and Corte de Pedra in Bahia, Brazil, where it is the principal vector of *L. braziliensis* (Franca et al, 1991).

In Opon, there is some evidence that forest replacement by cacao has a more positive effect on the abundance of *Lu. gomezi* than on *Lu. trapidoi*. In particular, both in the seasonal variation and in the nocturnal activity studies, the ratio of *Lu. trapidoi* : *Lu. gomezi* was significantly higher in the forest than in either the cacao or peridomicile. Such comparisons should be made with caution, as sandfly abundance in this study was only measured at ground level, and relative activity patterns at different heights above ground level will vary both with sandfly species and habitat. For example, in Panama the activities of *Lu. gomezi* and *Lu. trapidoi* were 17 and 14 times greater (respectively) in the canopy (28 m above ground) than at the ground level (Chaniotis et al, 1971b). Despite this caveat, the comparison of biting activity at ground level remains the most relevant for transmission as this is the parameter which directly determines the human infection rate (i.e. because humans are bitten at ground level).

The effect of deforestation on the abundance of both *Lu. gomezi* and *Lu. trapidoi* was previously investigated by Porter & DeFoliart (1981) in a 14 months study carried out in the east central region of the Antioquia Department, Colombia (approximately 150 Km. apart from the Opon focus; Figure 1.1). In the forest the geometric mean number (GM) of *Lu. trapidoi* was 0.91 compared with a GM of 0.05 for *Lu. gomezi* (ratio, 18:1) whilst in the cleared areas (areas without forest) a GM of *Lu. trapidoi* was 0.10 compared with a GM of 0.43 for *Lu. gomezi* (ratio, 4:1). Thatcher & Hertig (1966) in

Panama also reported an increase in the *Lu. gomezi* population in areas being rapidly cleared.

No direct comparisons have been previously reported of the relative preference for cacao and forest shown by *Lu. trapidoi* and *Lu. gomezi*. However, in La Tablada, Ecuador, the ratio of *Lu. gomezi*: *Lu. trapidoi* was greater in the forest than in the mixed coffee and cacao plantations (Mouchet et al, 1994); and *Lu. gomezi* was completely absent in a nearby coffee and cacao plantation in Paraiso Escondido, where *Lu. trapidoi* was relatively abundant. Without more data, it is fruitless to speculate why the pattern of habitat preference by these sandfly species in Opon is in direct contrast to the pattern observed on the Pacific foothills of Ecuador. However, recent publications have suggested that differences in the abundance of *Lu. gomezi* and *Lu. trapidoi* according to vegetation type may be related to speciation of these two sandfly species (Dujardin et al, 1996; Feliciangeli, 1997). Hence, investigation of the genetic heterogeneity of populations of these two species collected in cacao, primary forest and indoors in the Opon focus could be instructive.

b) Pasture and secondary forest

The effect of pasture on sandfly in the Opon focus was not directly measured because all collections were concentrated in the four

environments: forest, cacao, peridomicile and inside houses. However, the effect of the replacement of primary forest with either pasture and secondary forest can be inferred from the comparative study of endophagic activity. Surprisingly, both pasture and secondary forest had a negative impact on endophagic sandfly activity (Table 5.9). It is likely that pasture acts as a barrier to dispersal, as sandflies will have difficulty covering any significant distance from the edge of the forest or crops over grassland to reach a house. The negative impact of secondary forest could be associated with the total destruction of habitats following the burning of primary forest, which precedes the growth of secondary forest. The WHO in 1990 pointed out the benefits of the practice of clearing vegetation around houses as a valuable action in order to avoid vector/human contact; but in countries where deforestation is the main ecological problem this recommendation is not desirable. Secondary forest in other regions has previously been seen as a positive risk factor for indoor sandfly activity (e.g. *Lu. whitmani* in Sao Paulo: Forratini, 1954), probably due to different patterns of land use after deforestation or due to the different ecological requirements of sandfly species.

c) Effects of habitat change on sandfly fauna: conclusions

The differential effects of deforestation in the Opon focus on different sandfly species may explain the results of the regression analyses designed to detect associations between sandfly abundance and transmission rate. *Lu.*

gomezi abundance was correlated with the incidence rate during the follow-up study (both at the household and village level), indicating that this species currently plays an important vectorial role in Opon. In contrast, *Lu. trapidoi* abundance was related both with incidence and with cumulative prevalence, indicating that it has been an important vector for a number of years and that, unlike *Lu. gomezi*, the spatial pattern of its abundance has remained stable over time.

5.3.7 Sandfly activity in the domestic environment

a) Peridomestic activity

An accurate overview of the most active peridomestic sandflies in the Opon focus is not possible for the few collections made. But the evidence that is available from this study suggests that *Lu. hartmanni* and *Lu. trapidoi* predominate. Indeed, the proportion of *Lu. trapidoi* collected in the seasonal study was probably an underestimate of the relative abundance, as the time of collection (18 - 20 hours) is a period of relatively low activity for *Lu. trapidoi* compared to *Lu. gomezi* and *Lu. hartmanni* especially, and to *Lu. quasitowsendi* to some extent. Hence, *Lu. trapidoi* is certainly a potential vector in the peridomestic environment.

b) Endophagic behaviour

According to the results of the seasonal and nocturnal studies, it is clear that *Lu. quasitownsendi* is characterised by a relatively high predisposition to enter houses, whereas *Lu. hartmanni* is characterised by relatively low endophagy. *Lu. gomezi* and *Lu. trapidoi* are characterised by similar, and intermediate, levels of endophagy. However, these behavioural attributes are not reflected in the relative activity of the different sandflies inside houses, as determined by the indoor CDC light trap survey, presumably because of a significant difference in the population sizes of the four species. From the indoor CDC light trap survey, *Lu. trapidoi* predominates, followed by *Lu. gomezi* and *Lu. hartmanni*, and *Lu. quasitownsendi* is relatively rare. The relative risk of being bitten indoors by *Lu. trapidoi*, in comparison to *Lu. gomezi*, is also probably underestimated by this survey, as the CDC light traps, tend to over-represent *Lu. gomezi* (in comparison with *Lu. trapidoi*). Hence, there is evidence that *Lu. trapidoi* is the most common indoor species in the Opon focus, followed by *Lu. gomezi*; and these two species are clearly capable of transmitting cutaneous leishmaniasis to families sleeping inside their houses at night.

Seasonal changes in endophagic activity were also monitored, on the basis that the behavioural decision to enter houses for a blood meal may depend on outdoor conditions which varied with season. For example, Le

Pont et al (1994a) reported that *Lu. trapidoi* and *Lu. gomezi* increased their indoor abundance during the wet season in two different villages in Ecuador. In the Opon focus, endophagic activity of three species (*Lu. trapidoi*, *Lu. quasitowsendi* and *Lu. hartmanni*) all appeared to peak twice: in June-July and between October - December. The latter peak coincided with the peak period of human leishmaniasis cases (see Chapter 4). However, no significant correlations were detected between endophagic activity and climatic conditions.

c) Dispersal to the domestic environment from the surrounding habitat

For *Lu. gomezi* in Opon, there is circumstantial evidence that breeding places occur in the peri-domestic habitats, with adult females taking advantages of the high density of blood sources inside and around houses (humans and domestic animals). In contrast, the breeding places of *Lu. trapidoi* appear to be further from houses, inside the cacao crops fields and, probably close to the remaining natural trees. *Lu. trapidoi* has previously been reported to use a broad breeding niche, on the basis of an emergence cage survey on the open forest floor in Panama (Rutledge & Ellenwood, 1975), and immature stages of *Lu. trapidoi* have been found in the soil between buttresses, in the soil from animal burrows and amongst dead leaves on the forest floor (Hanson, 1961).

In Opon, there is also circumstantial evidence that the dispersal activity of *Lu. trapidoi* tends to be over greater distances than that of *Lu. gomezi*. No previous studies appear to have analysed dispersal of these sandflies towards houses. Instead, most previous dispersal studies (using mark - recapture) were carried out within the forest. In a rain forest in Panama, circa 20,000 sandflies were marked and released at ground level and on a canopy platform (Chaniotis et al, 1974): 90% of the recaptured flies were recovered within 57 m of their release site. Consistent with this result, 74% of recaptured sandflies were found within a distance of 76 m from the release point in a Colombian coffee plantation (Alexander, 1987).

The distance of sandfly attraction to humans has rarely been measured. However, in Peru (Davies et al, 1995c) a single human bait was apparently unable to attract *Lu. verrucarum* (a known anthropophilic species) even from very short distances (5 m). In Opon, however, it appears that sandflies may be attracted from greater distances towards houses (i.e. up to 300 m for *Lu. trapidoi*), probably because there are more abundant sources of blood, and a strong odour plume from both domestic animals and humans.

5.3.8 Nocturnal activity of sandflies

Although the temporal patterns of nocturnal activity were only studied on three nights, the results for the five most abundant species collected in this study (*Lu gomezi*, *Lu yuilli*, *L hartmanni*, *L trapidoi*, *L quasitownsendi*) provide convincing evidence for interspecific differences. This is perhaps surprising given the results of previous long-term studies on nocturnal sandfly activity which have demonstrated considerable variability in nocturnal activity patterns between nights (e.g. Porter & Defoliart (1981) in Antioquia, Colombia; Feliciangeli (1997) in Miranda, Venezuela; Villaseca et al (1993) in Ancash, Peru). For example, the peak periods of activity for both *Lu. trapidoi* and *Lu. hartmanni* were shown to be very variable throughout the year in Antioquia, Colombia (Porter & DeFoliart, 1981); and similar night-to-night variability was detected in Venezuela for a number of species, including *L. gomezi*, during a three year study in Venezuela (Feliciangeli, 1997). But in both studies, there was no clear seasonal pattern to explain the night-to-night variability in nocturnal activity, and there were some consistent trends which distinguished the species. In contrast, in the Peruvian Andes (Villaseca et al, 1993), seasonal changes in the peak of activity of both *Lu. verrucarum* and *Lu. peruensis* were identified and these coincided with seasonal changes in ambient temperature (during some season the outdoor night temperature was 8°C and 16-22°C indoor). Hence, the absence of any clear seasonal changes in the nocturnal activity patterns of sandflies in either Antioquia or

Miranda may be explained by the relatively constant temperature throughout the year. Opon, too, has a relatively constant temperature throughout the year (Figure 2.10 and Figure 2.11), and major seasonal changes in the nocturnal activity patterns are probably not to be expected.

Lu. trapidoi was the only one of the five species studied in Opon which demonstrated peak activity after midnight (Figure 5.5), when the inhabitants are sleeping. Given the evidence of endophagic activity (see Section 5.3.7), this late peak of activity is consistent with the putative role of *Lu. trapidoi* as the major vector in this focus. In contrast, *Lu. yuilli*, in particular, and *Lu. hartmanni*, to a lesser extent, demonstrated peak activity at the time when the inhabitants tend to concentrate in the dwellings before bed time. The activity of *Lu. gomezi* also peaked early in the evening, but significant activity continued throughout the night, as it did for *Lu. quasitownsendi*. The nocturnal pattern of *Lu. quasitownsendi* in Opon is the first published description of this species. But for the other four species, studies of nocturnal activity have been reported previously, and their results are compared below with the patterns observed in Opon.

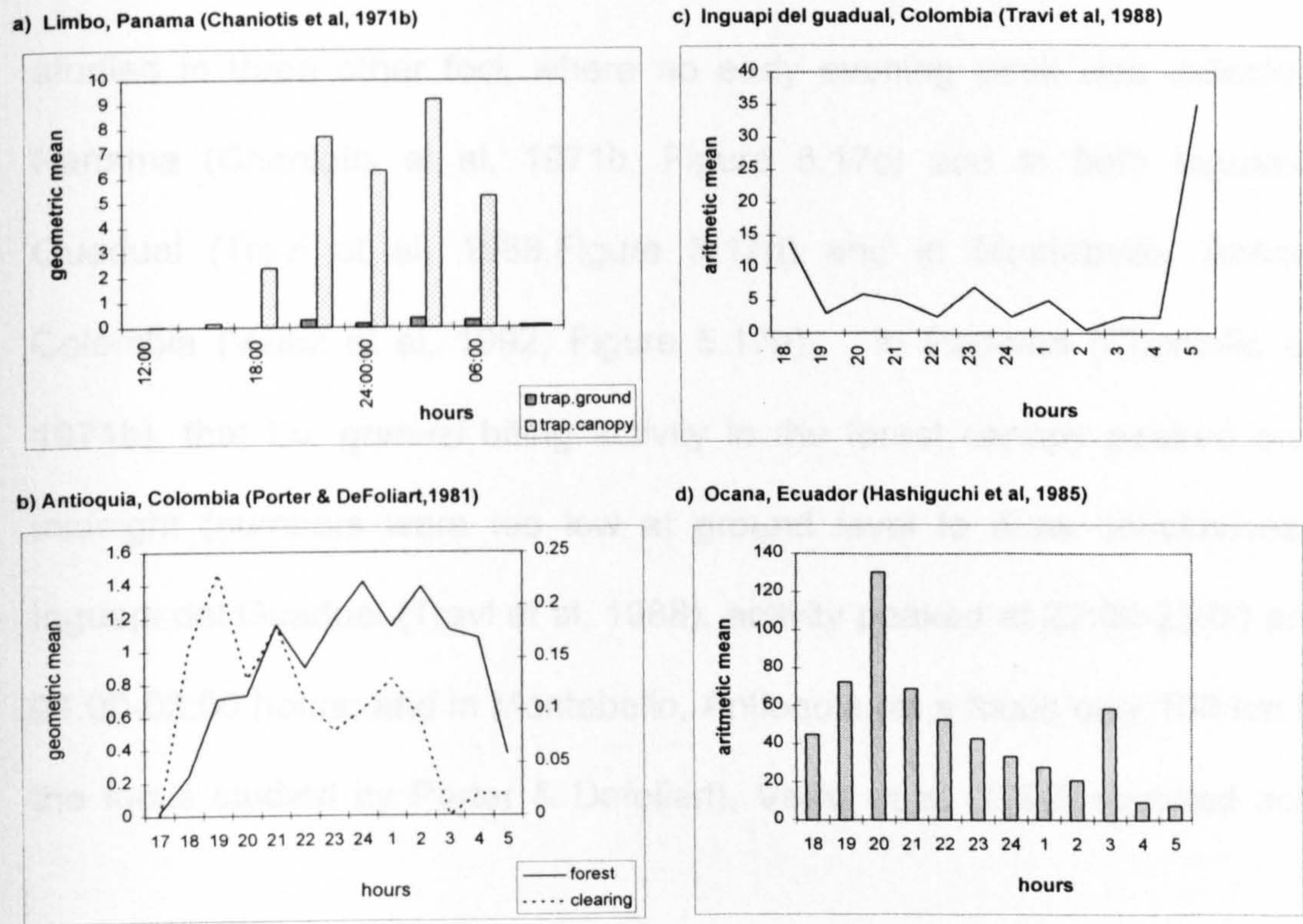
a) *Lutzomyia trapidoi*

The nocturnal biting activity of *Lu. trapidoi* in the Opon focus differed significantly from that of the other anthropophilic species, with a clearly

defined peak in the second half of the night (02:00-03:00 hours) (Figure 5.5). A similar pattern was described by *Lu. trapidoi* both at ground level and in the canopy in a forest site in Panama (Figure 5.16a) (Chaniotis et al, 1971b), as well as at ground level in a forest site in Antioquia, Colombia (Figure 5.16b) (Porter & DeFoliart 1981), which is ca. 150 km from the Opon focus in the same interandean valley (Figure 1.1). However, in the Antioquia site, biting activity of *Lu. trapidoi* in clearances (i.e. sites of deforestation) showed a distinct peak early in the evening between 19.00 and 20.00 hours (Figure 5.16b). This difference in behaviour could either be due to environmental pressures or genetic differences in sandfly populations which are adapted to the different habitats. In Peru, for example, the nocturnal biting activity of Andean sandflies showed quite distinct patterns inside and outside houses, possibly due to the temperature differential (Villaseca et al, 1993). However, a genetic basis to behavioural differences between and within *Lu. trapidoi* populations is a real possibility, following the finding in La Tablada and Paraiso Escondido, Ecuador, of two genetically distinct sympatric populations of *Lu. trapidoi*, as defined by multilocus enzyme electrophoresis (Dujardin et al, 1996). These cryptic species are thought to demonstrate differences in their intradomiciliary activity, and it is conceivable that they could also vary in their nocturnal activity patterns (although no data are yet available to test this hypothesis).

Again, it is unclear whether genetics or the environment is responsible for the distinct nocturnal activity patterns detected in the *Lu. trapidoi* populations of the Pacific coast lowlands of Colombia and Ecuador. Both in Inguapi del Guadual, Colombia (Figure 5.16c) (Travi et al, 1988); and in Ocana, Ecuador (Figure 5.16d) (Hashiguchi et al, 1985b), *Lu. trapidoi* activity peaks twice: in the evening and then again in the early morning. Clearly, further work is needed to disentangle the various factors which could determine inter -or intra- population differences in nocturnal activity. In particular, it would be beneficial to carry out a genetic characterisation of the *Lu. trapidoi* populations whose behaviour has already been studied.

Figure 5.16 Nocturnal activity patterns of *Lu. trapidoi* previously reported in Central and South America



b) *Lutzomyia gomezi*

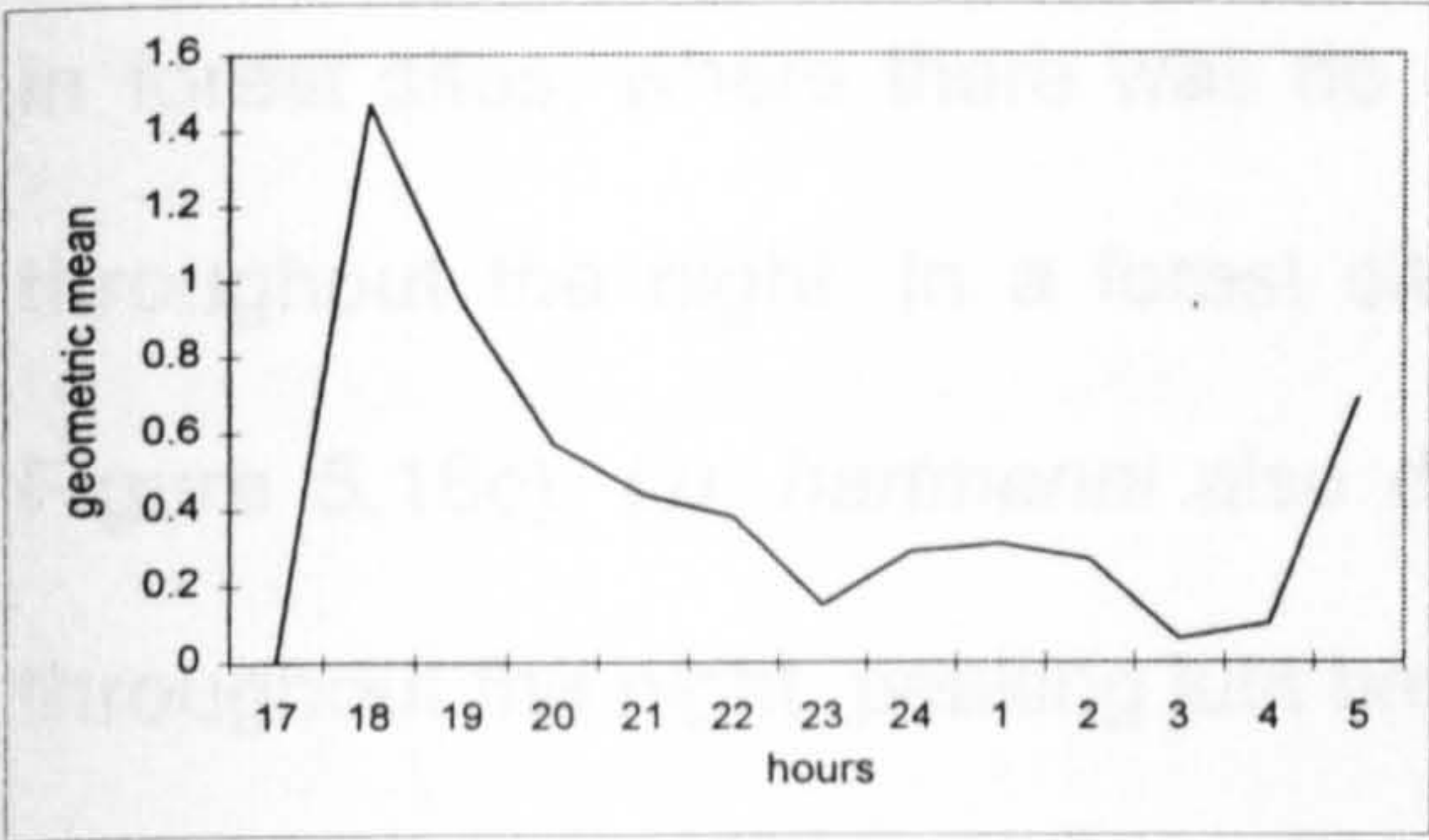
In the Opon focus, *Lu. gomezi* biting activity peaks early (19:00-20:00 hours) but continues at a significant rate throughout the night (Figure 5.3). An early evening activity peak between 18:00 and 22:00 hours was also detected in *Lu. gomezi* populations in forest sites in Antioquia, Colombia (Porter & DeFoliart, 1981, Figure 5.17a) and in Miranda, Venezuela (Felicangeli, 1997, Figure 5.17b). But the Opon population differed from these two in the extension of the biting activity throughout the night. Also, a smaller second activity peak (from 05:00 to 06:00 hours) was uniquely observed in the Antioquian population.

However, the nocturnal activity patterns of *Lu. gomezi* in the Opon focus contrast most strongly with the patterns observed in the populations studied in three other foci, where no early evening peak was detected: in Panama (Chanotis et al, 1971b, Figure 5.17c) and in both Inguapi del Guadual (Travi et al, 1988, Figure 5.17d) and in Montebello, Antioquia, Colombia (Velez et al, 1992, Figure 5.17e). In Panama (Chanotis et al, 1971b), that *Lu. gomezi* biting activity in the forest canopy peaked around midnight (numbers were too low at ground level to draw conclusions); in Inguapi del Guadual (Travi et al, 1988), activity peaked at 22:00-23:00 and at 01:00-02:00 hours; and in Montebello, Antioquia (at a focus only 100 km from the focus studied by Porter & Defoliart), Velez et al (1992) reported activity

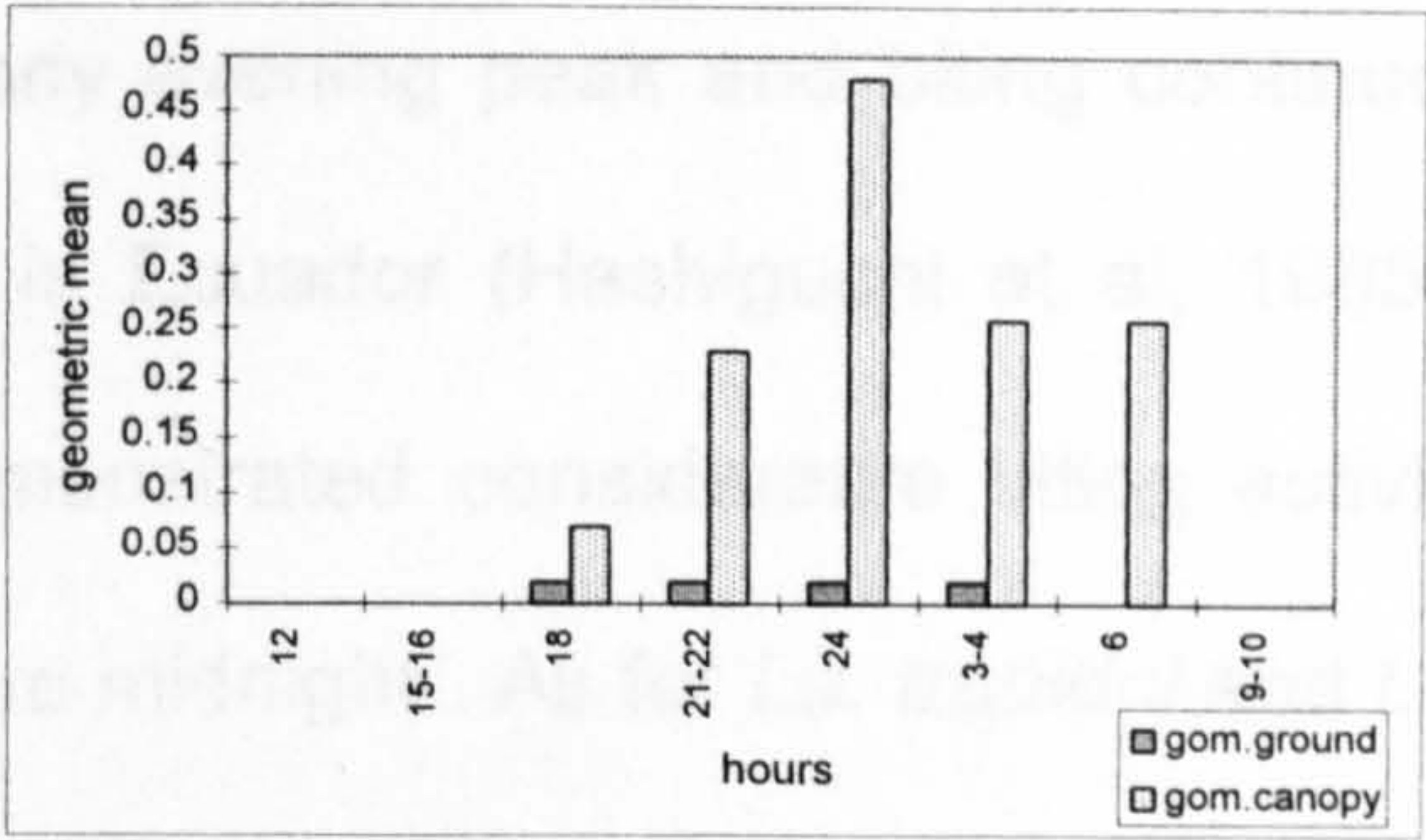
peaks at 22.00-23.00 and at 03-04.00 hours. As for *Lu. trapidoi*, the cause of these different patterns of biting activity could be related either to variable environmental conditions or genetic variation (Feliciangeli, 1997).

Figure 5.17 Nocturnal activity patterns of *Lu. gomezi* previously reported in Central and South America

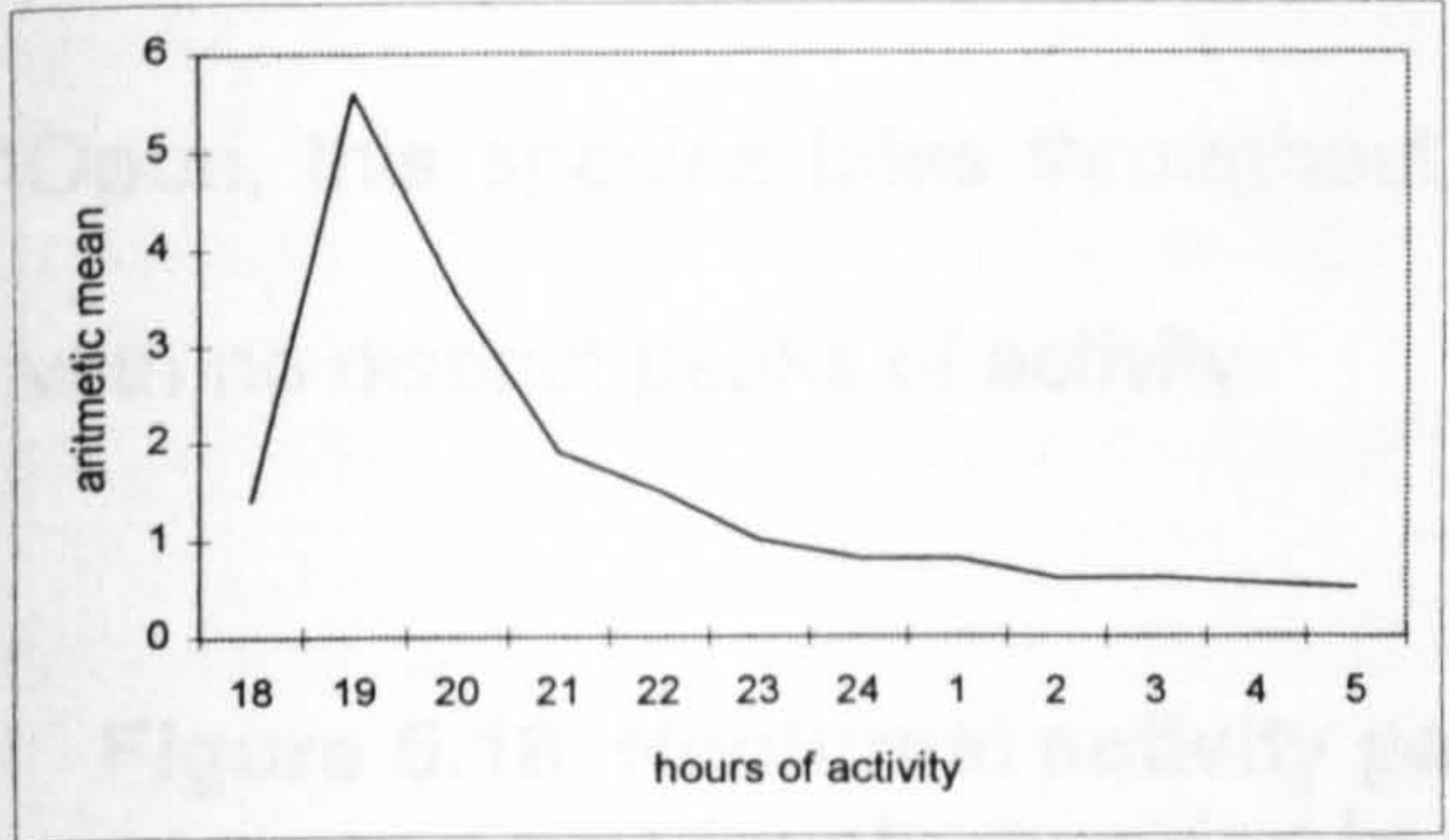
a) Antioquia, Colombia (Porter & DeFoliart, 1981)



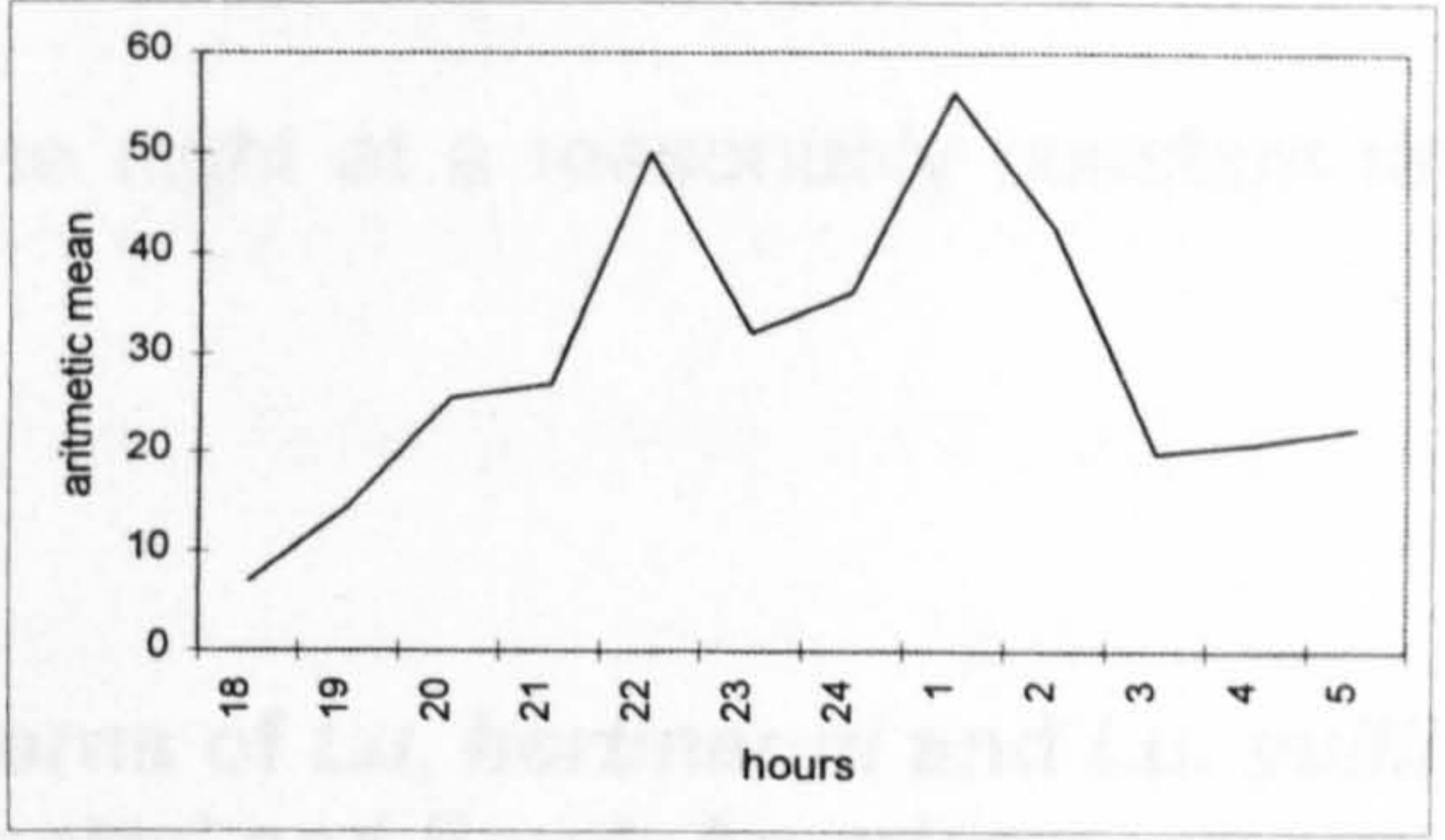
c) Limbo, Panama (Chaniotis et al, 1971b)



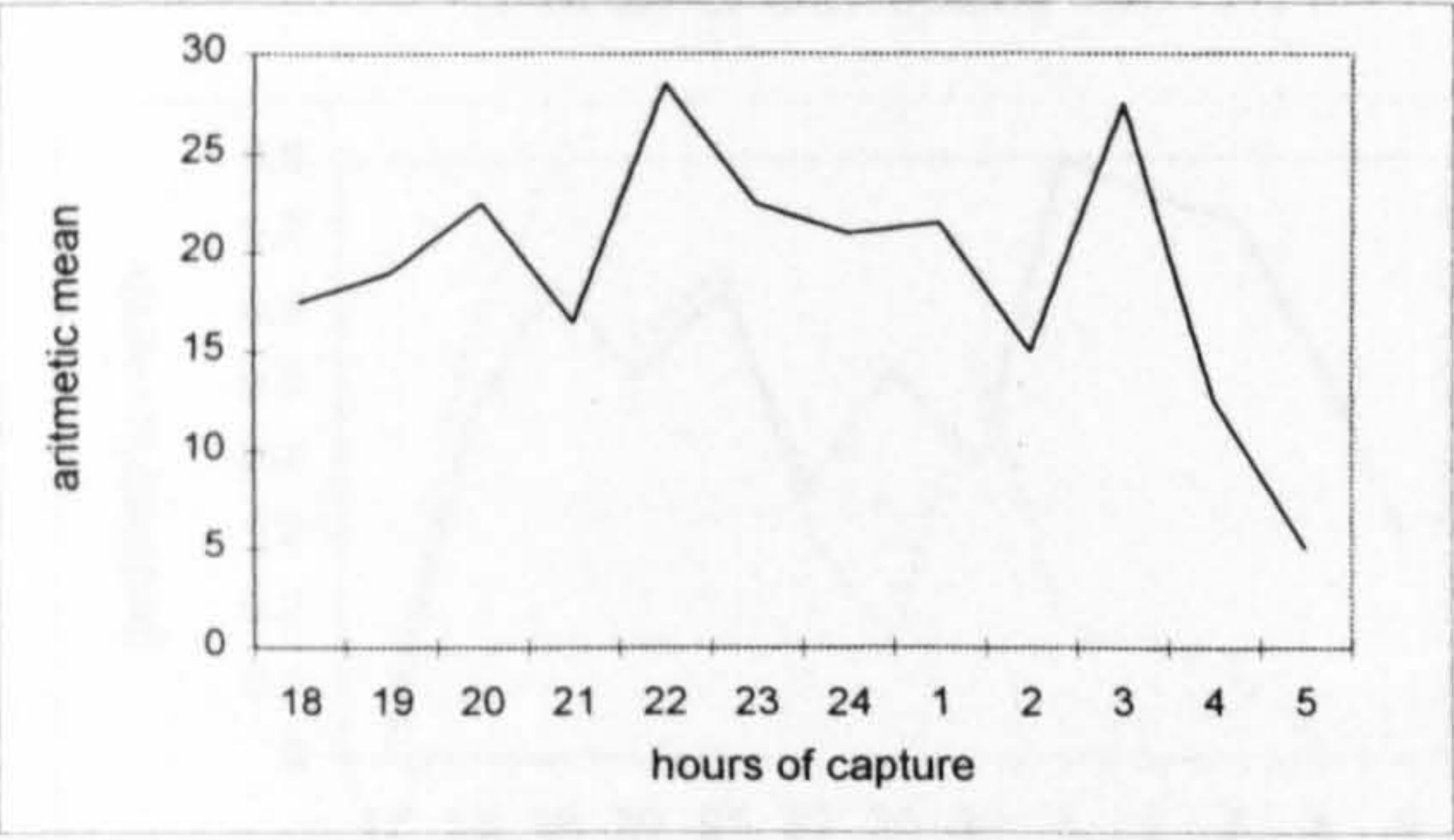
b) Miranda, Venezuela (Feliciangeli, 1987)



d) Inguapi del guadual, Colombia (Travi et al, 1988)



e) Montebello Antioquia, Colombia (Velez et al, 1992)

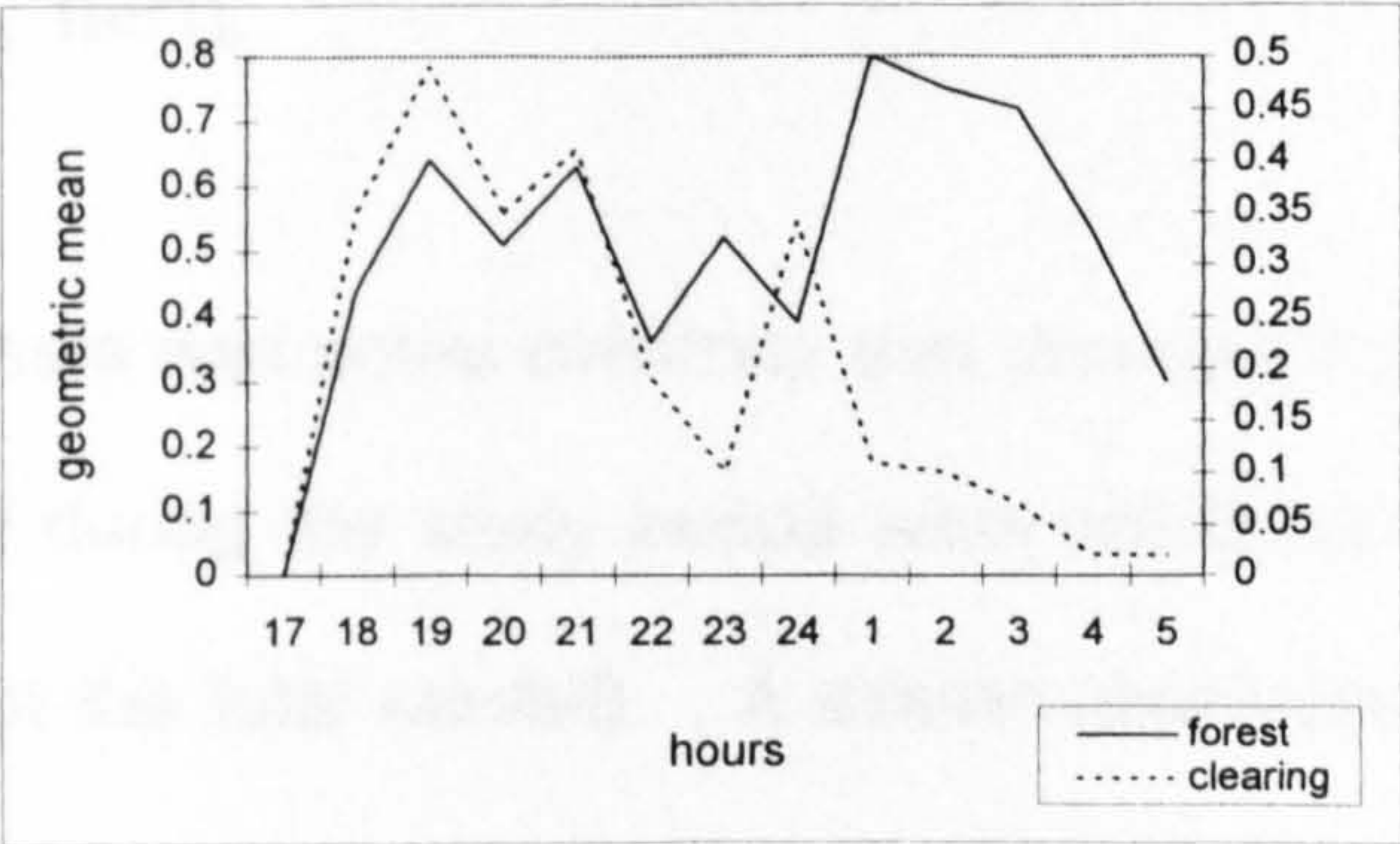


c) *Lu. hartmanni*, *Lu. yuilli* and *Lu. quasitowsendi*

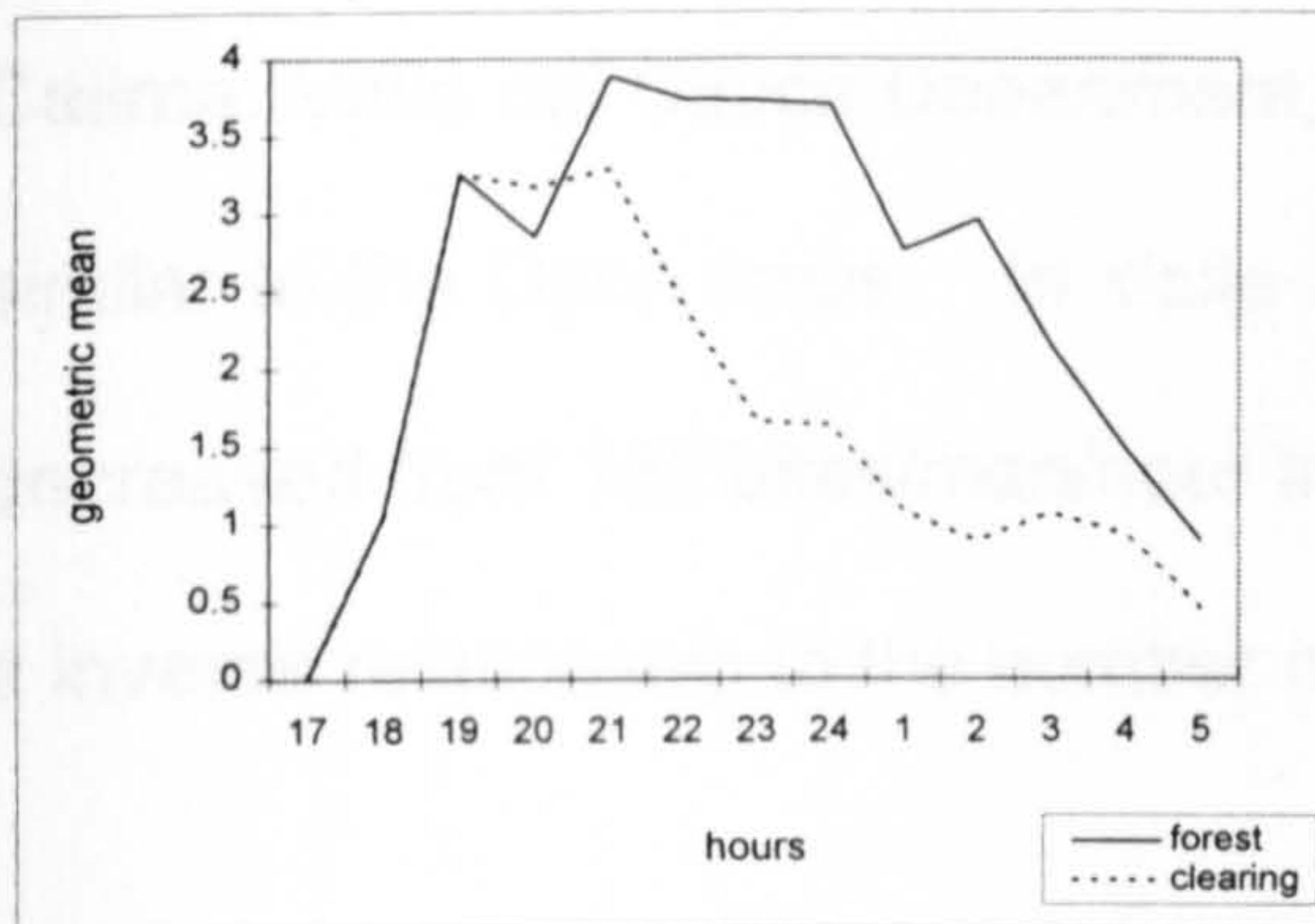
The early evening activity peaks detected for both *Lu. hartmanni* and *Lu. yuilli* in the Opon focus (Figure 5.1 and Figure 5.2) are not dissimilar to the patterns shown by populations of these species studied in clearances in Antioquia, Colombia (Porter & DeFoliart, 1981, Figure 5.18. a,b). However, in Antioquia, both these species demonstrated very different activity patterns in forest sites, where there was no early evening peak and biting continued throughout the night. In a forest site in Ecuador (Hashiguchi et al, 1985b, Figure 5.18c), *Lu. hartmanni* also demonstrated considerable biting activity throughout the night, peaking just before midnight. As for *Lu. trapidoi* and *Lu. gomezi*, the explanation for these differences is not yet clear. There are no previous reports of the nocturnal activity patterns of *Lu. quasitowsendi*. In Opon, this species bites throughout the night at a reasonably constant rate with no distinct peaks of activity.

Figure 5.18 Nocturnal activity patterns of *Lu. hartmanni* and *Lu. yuilli* previously reported in Central and South America

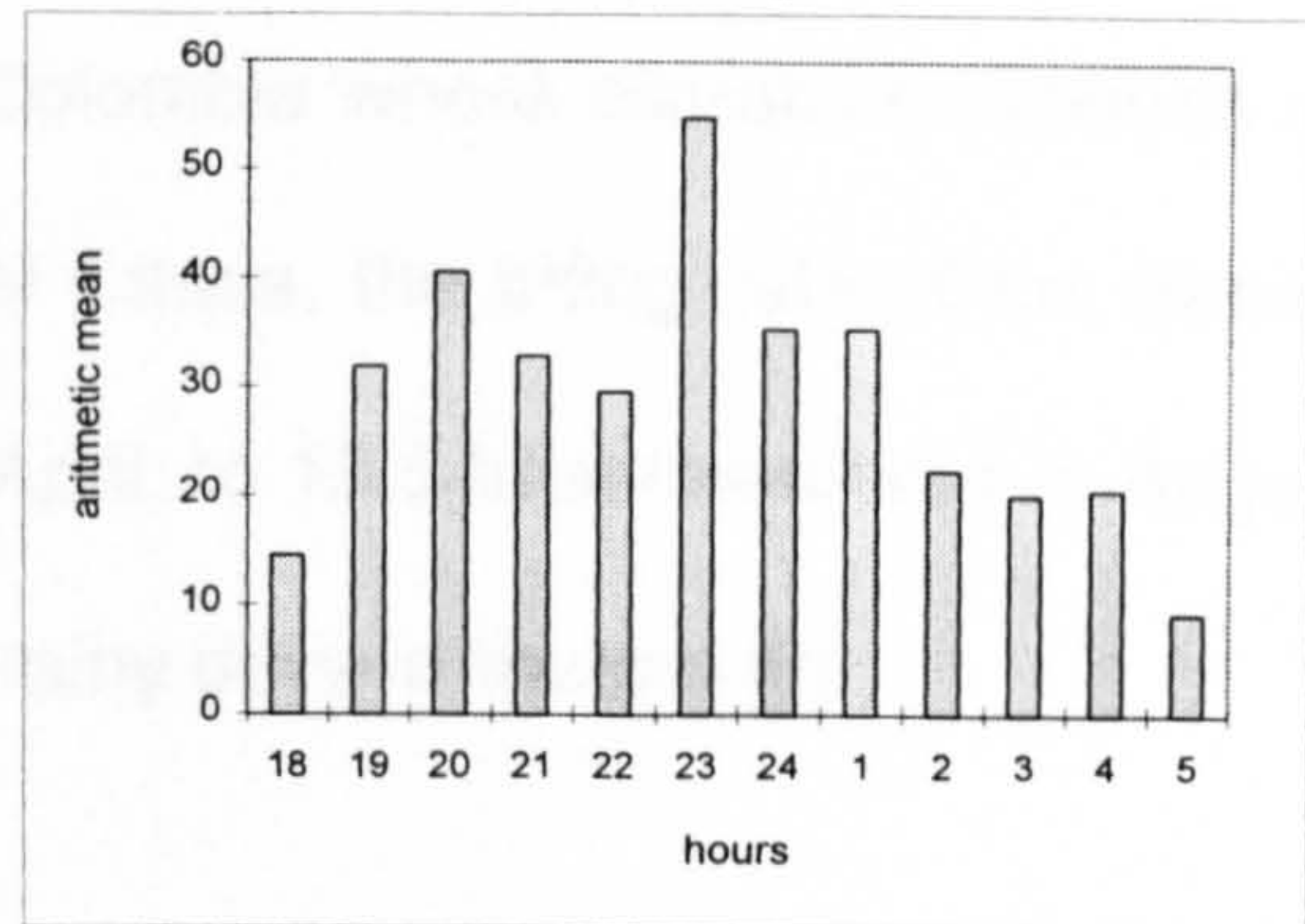
a) *Lu yuilli*, Montebello, Antioquia, Colombia
(Porter & DeFoliart, 1981)



b) *Lu hartmanni* Montebello, Antioquia, Colombia (Porter & DeFoliart, 1981)



c) *Lu hartmanni*, Ocaña, Ecuador (Hashiguchi et al, 1985)



5.3.9 Seasonal variation study

The seasonal patterns of sandfly species in Opon (such as they were) were not clearly associated with changes in the climatic conditions, either during the same month as the sandfly collection or in any of the three previous months. This lag period was chosen on the basis of lab studies which show that sandfly species complete their life cycle within this period; for example: *Lu. trapidoi* take 33 to 47 days to reach the adult stage (Johnson & Hertig, 1961; Chaniotis, 1975); and the total time between oviposition and adult emergence averages about 34 days for *Lu. gomezi* (Johnson & Hertig, 1961).

However, there was some evidence that changes in the abundance of *Lu. quasitowsendi* during the study period were related to the frequency of rainy days (but not the total rainfall). A similar observation was previously

made by Loyola et al (1988) in a study carried out in a botanic garden in Bajo Calima, Valle del Cauca Department, Colombia where climatic conditions are similar to the Opon focus. In Valle del Cauca, the biting rate of *Lu. trapidoi* decreased from 152 bites/man/hour in April, to 13.5 bites/man/hour in August, a inverse relationship to the number of rainy days in the month.

In the majority of previous studies carried out in tropical countries, the seasonal variation in the abundance of sandfly species had been related with the total rainfall pattern, rather than to humidity or temperature which are fairly constant throughout the year. Hence, in previous studies it is common to find the description of “wet” or “dry” species (Christensen et al, 1983; Feliciangeli, 1997). The Opon focus seasonal data are discussed below in relation to previous seasonal variation studies by rainfall pattern, type of vegetation, and collection methods: **Colombia**: Porter and DeFoliart (1981), Travi et al (1988), Alexander et al (1992), and Velez et al (1992); **Venezuela**: Feliciangeli (1987b); **Ecuador**: Le Pont et al (1994a); and **Panama**: Chaniotis et al (1971b) (Table 5.14). As for nocturnal activity, in the following sections, results of seasonal patterns are discussed by species.

Table 5.14 Seasonal variation of sandflies in Central America and Andean countries

rainfall patterns	# of rain eak	Author	Ctry year	rainfall (mm) total (range)	sampling		sandfly species		peaks of sandflies in each climatological period (month)				veget. type @
					method*	freq / durat.	trap.	gom.	dry	early wet	late wet	random	
high rainfall no dry months	1	Porter	Col/81	5,278 (192 - 700)	ML	2/month/ 1year	X	few	-	-	-	T-H	1
	1	Travi	Col/87	3,075 (175 - 450)	LT + ML	6 visits/ 1 year	X	X	H (N)	T (J-F)	G (AG-O)	-	1
	2	Alexander	Col/92	1,800 (90 - 300)	TT+ML+LT	2/month/ 3 y.	no	X	-	-	-	G	2
	2	Opon-focus	Col/97	2,153 (50.8 - 368)	ML	2/month/ 1 year	X	few	-	-	-	T-H	3
high rainfall relat. dry month	1	Chaniotis	Pan/71	2,589 (0 - 110)	LT + TT	4/month/16 mon.	X	X	G (J-A)	T (M-AG)	T (S-D)	-	1
	1	Le Pont	Ecu/94	3,215 (0 - 700)	LT+ML	1/month/ 1 year	X	X	G (O)	T (D-F)	G(M-A)	-	2-3
low rainfall	1	Feliciangeli	Ven/87	700 (1 - 108)	ML+SH	2/month/ 1 year	no	X	-	-	G (S-O)	-	4
	1	Velez	Col/92	678 (4 - 44)	ST	every day/ 2.5y.	no	X	-	G (J-O)	-	-	2

* ML= man-landing; TT= aspiration from tree trunks; SH= Shannon traps; ST= stiky traps; LT= light traps

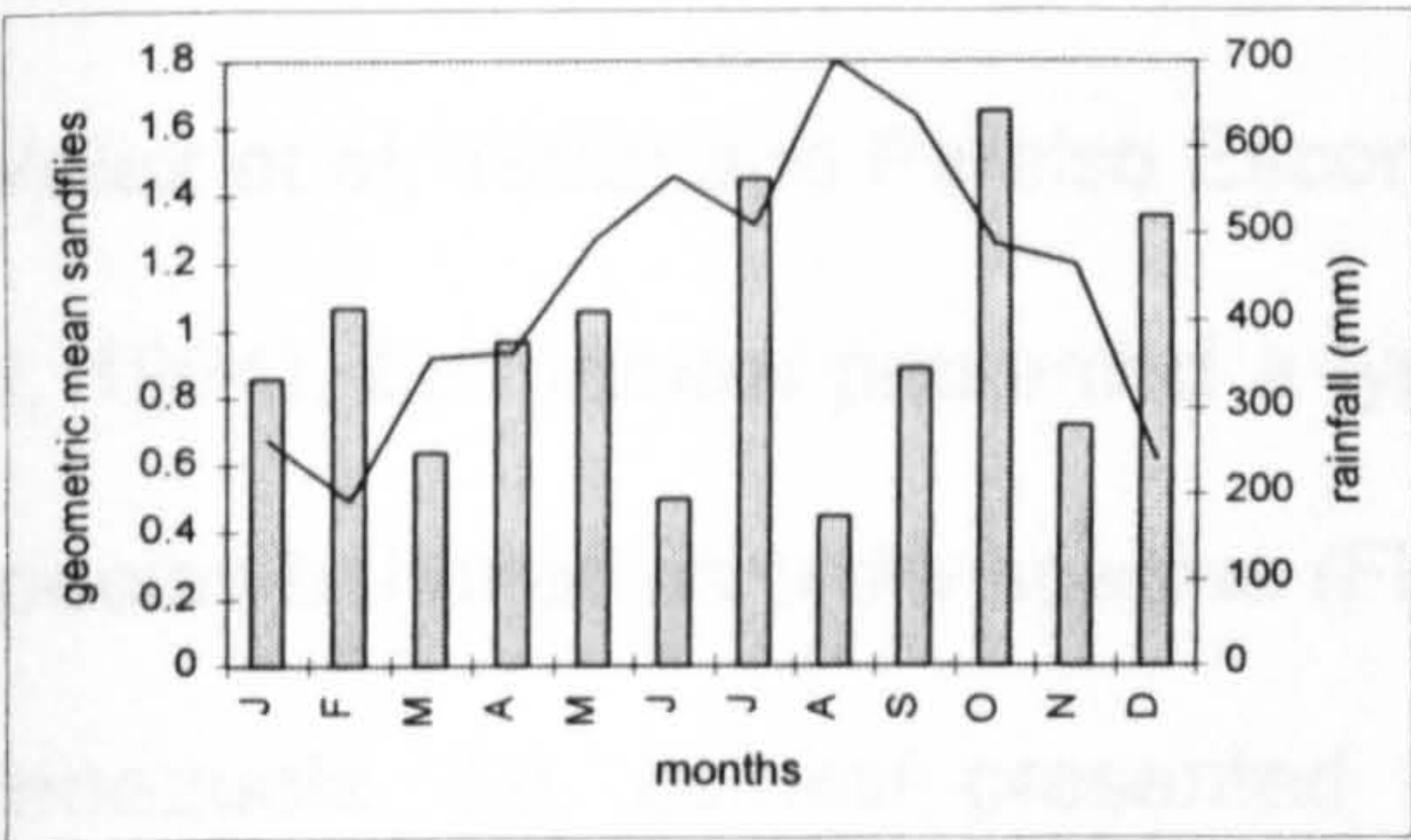
@ 1 = tropical rain forest; 2 = coffee grown area with large trees; 3 = cacao grown area with large trees; 4 = agriculture area with large trees

a) *Lu. trapidoi*

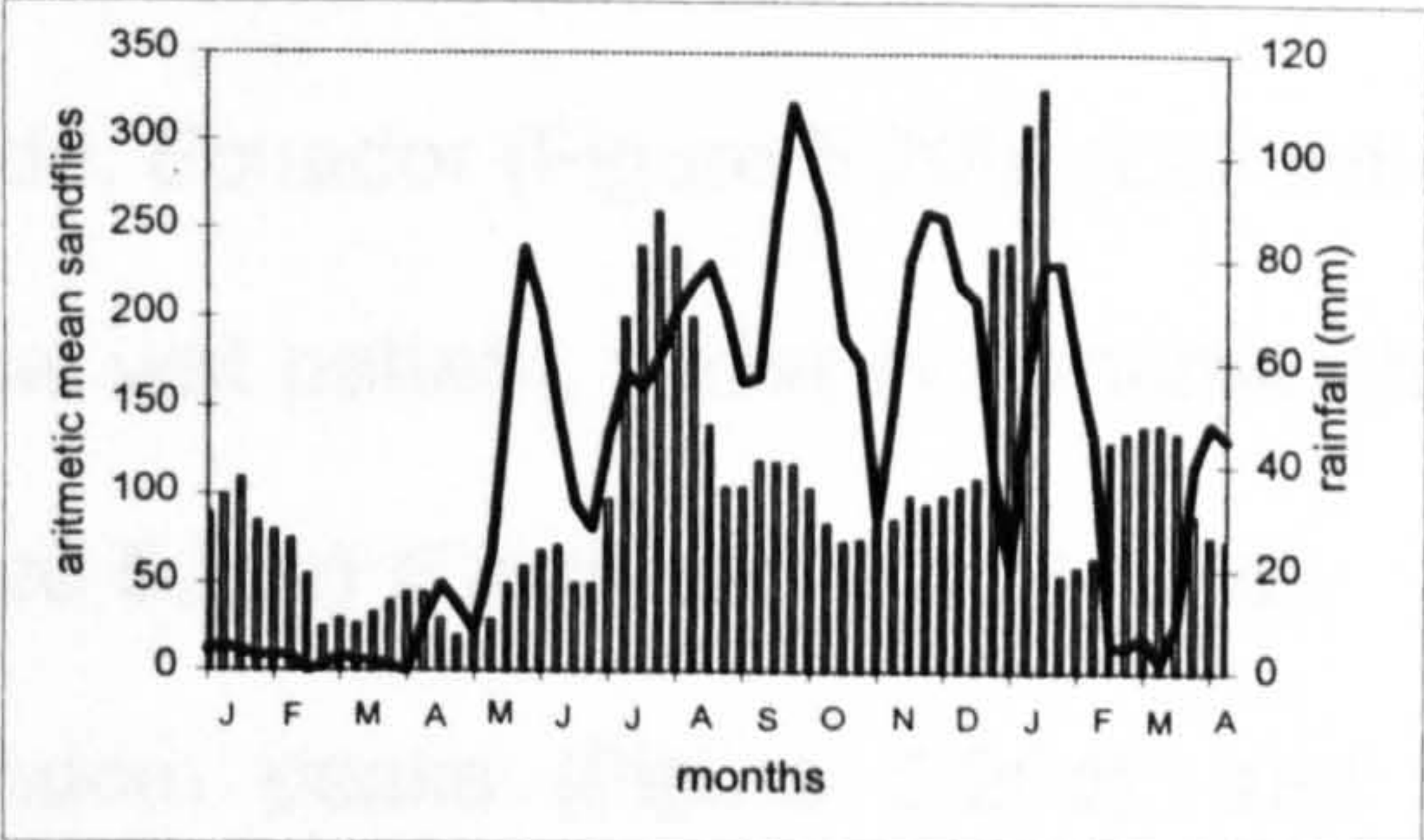
In the Opon focus study, this species presented random peaks of abundance during the whole year with no significant seasonal trend (Figure 5.13), similar to the pattern presented for this species in the nearest study site in Antioquia (Porter & DeFoliart, 1981, Figure 5.19) where seasonal changes were described as erratic, but in another Colombian study (Travi et al, 1987, Figure 5.19) *Lu. trapidoi* decreased in proportion from January to October, independently of the bimodal cycle of rain presented in that region.

Figure 5.19 Seasonal variation studies of *Lu. trapidoi* previously reported in Cental and South America

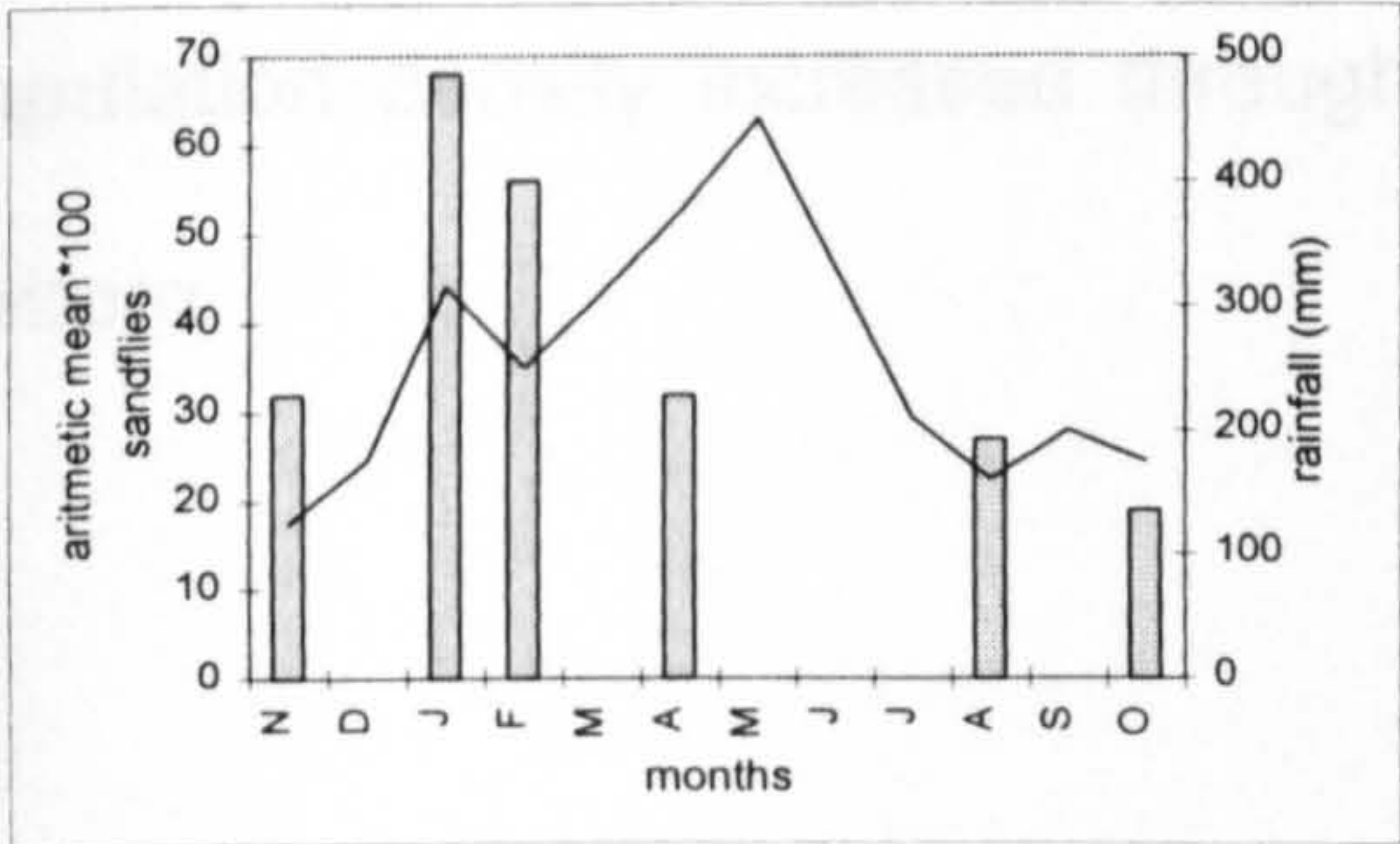
a) Antioquia, Colombia (Porter & DeFoliart, 1981)



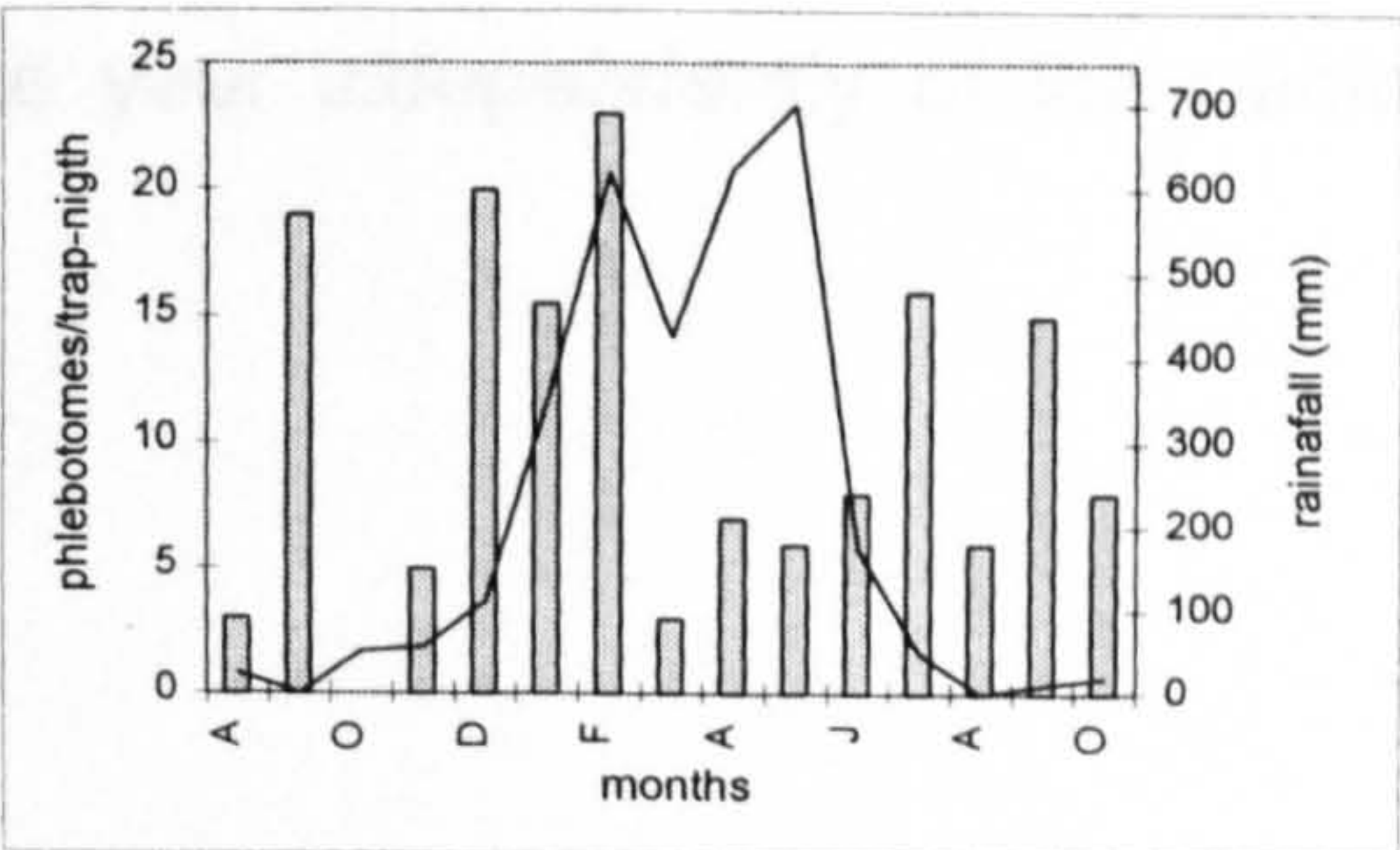
c) Limbo, Panama (Chaniotis et al, 1971)



b) Inguapi del guadual, Colombia (Travi et al, 1988)



d) Paraiso Escondido, Ecuador (Le Pont et al, 1994)



Bars represents sandflies abundance and solid line the rainfall patterns

By contrast in both, in Panama (Figure 5.19c) and Ecuador (Figure 5.19d), *Lu. trapidoi* presented a wet season pattern, with population peaks in the late and in the early wet periods respectively (Chaniotis et al, 1971b; LePont et al, 1994) (Table 5.14 and Figure 5.19),

b) *Lu. gomezi*.

In the Opon focus, the number of individuals collected of *Lu. gomezi* were too low to draw conclusions about their seasonal pattern. The seasonal variation of *Lu. gomezi* in the canal zone in Panama presented a typical dry pattern, whilst in Colombia, Ecuador and Venezuela *Lu. gomezi* behaved as a wet species (Figure 5.21). However, previous studies of seasonality have shown inconsistent results; i.e. both in Antioquia, Colombia (Figure 5.20a) (Velez et al, 1992) and Paraiso Escondido, Ecuador (Figure 5.20b) (LePont et al, 1994), *Lu. gomezi* presented a typical wet pattern, whilst in Panama, this species behaved as a dry species (Figure 5.20c) (Chaniotis et al, 1971b). In Venezuela, *Lu. gomezi* presented random peaks (Figure 5.20d), and in Inguapi del Guadual, Colombia (Figure 5.20e) (Travi et al, 1988) the population density increased through the year independently of the rainfall pattern.

c) *Lu. hartmanni*

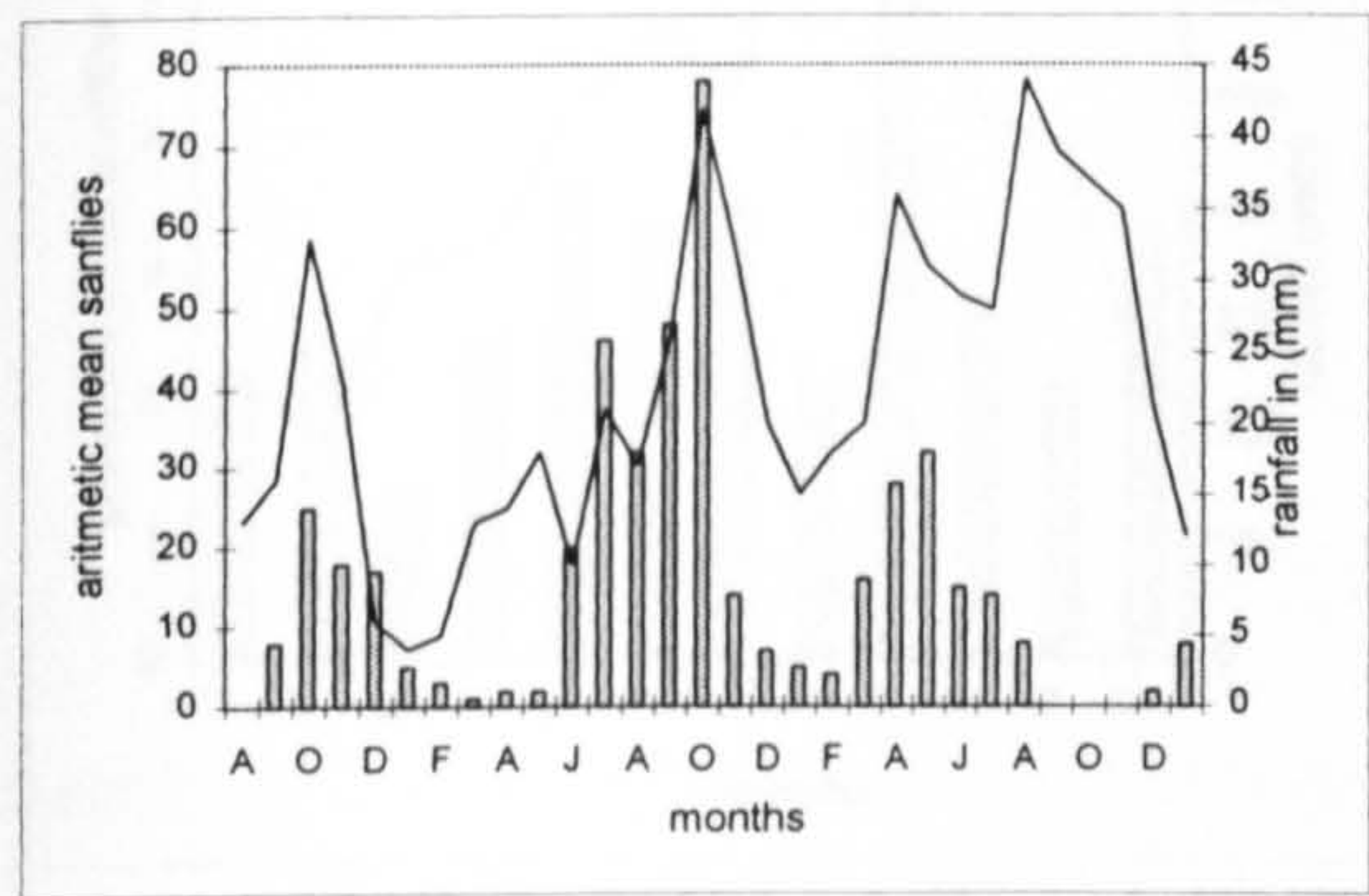
The abundance of this species decreased significantly during the 11 months of the Opon study (see Section 5.2.6). The reduction during the year reflects presumably a real event, and is not due to bias, because different patterns were observed for the other species, notably *Lu. trapidoi* which remained equally active throughout the whole year. In previous studies (Table 5.14), *Lu. hartmanni* did not present any particular seasonal pattern: in Antioquia (Porter & DeFoliart, 1981, Figure 5.21a) the population changes were mild during the study ; and in Tumaco (Travi et al, 1988) there was a peak at the beginning of the study in the dry season, but this species was rarely collected in all of the later samples surveys (Figure 5.21b)

d) *Lu. quasitowsendi*

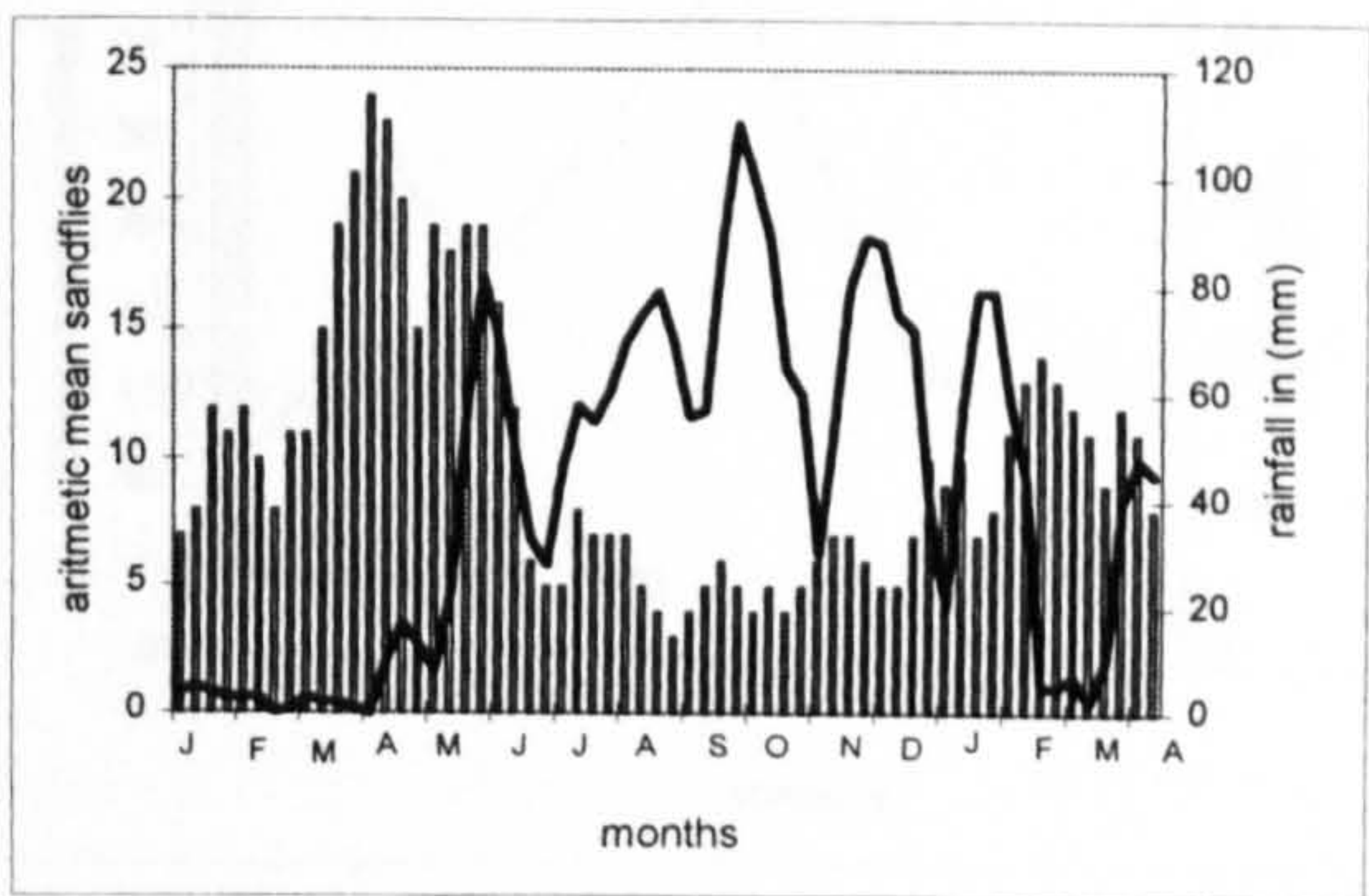
Due to the restricted distribution of this species, this report is the first analysis performed of the seasonality of this species, which appeared to be similar to that of *Lu. hartmanni*. The abundance of *Lu. quasitowsendi* decreased throughout the study period, and there was a slight suggestion that abundance correlates presumably with the frequency of rainy days.

Figure 5.20 Seasonal variation studies of *Lu. gomezi* previously reported in Cental and South America

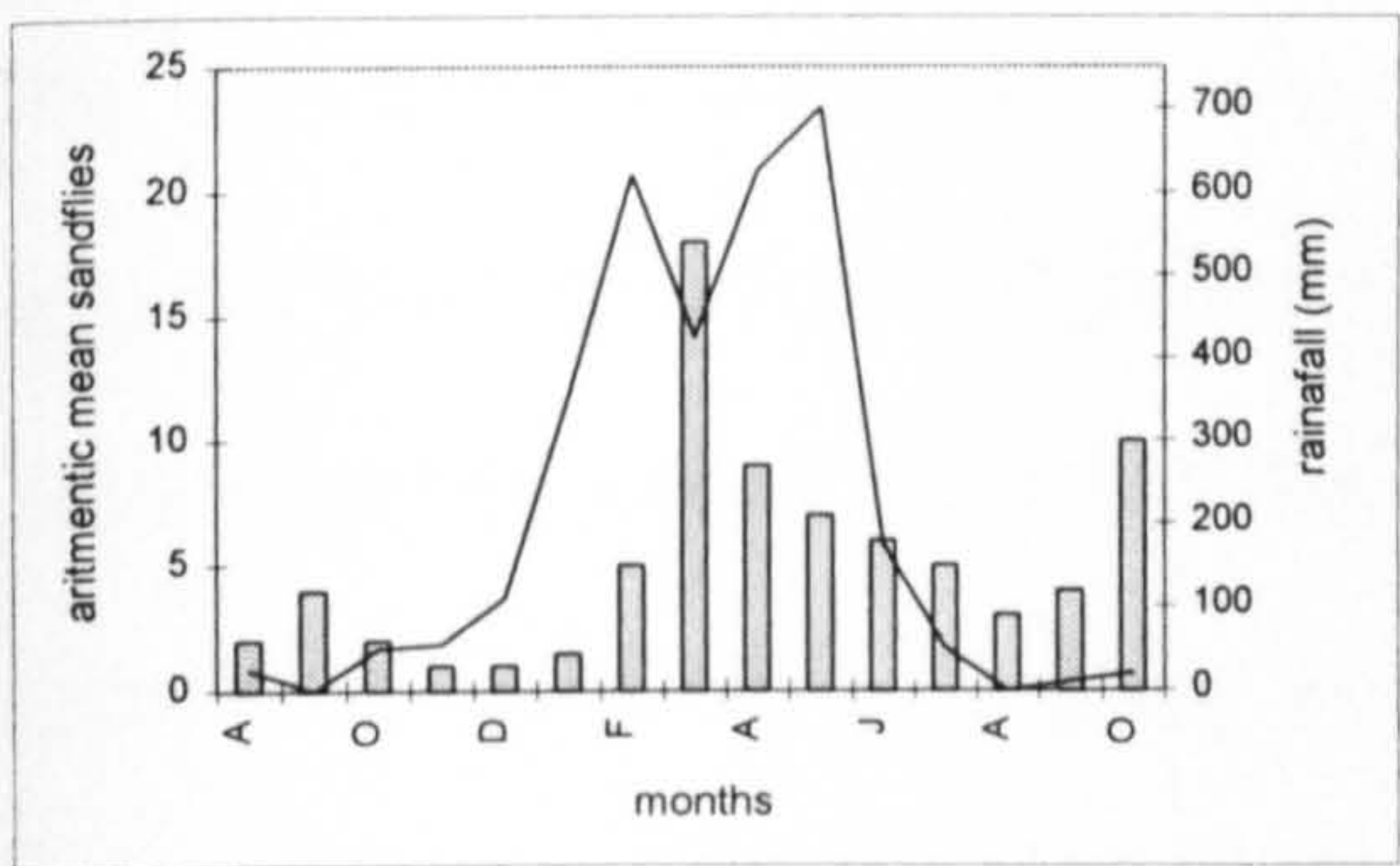
a) Montebello Antioquia, Colombia (Velez et al, 1992)



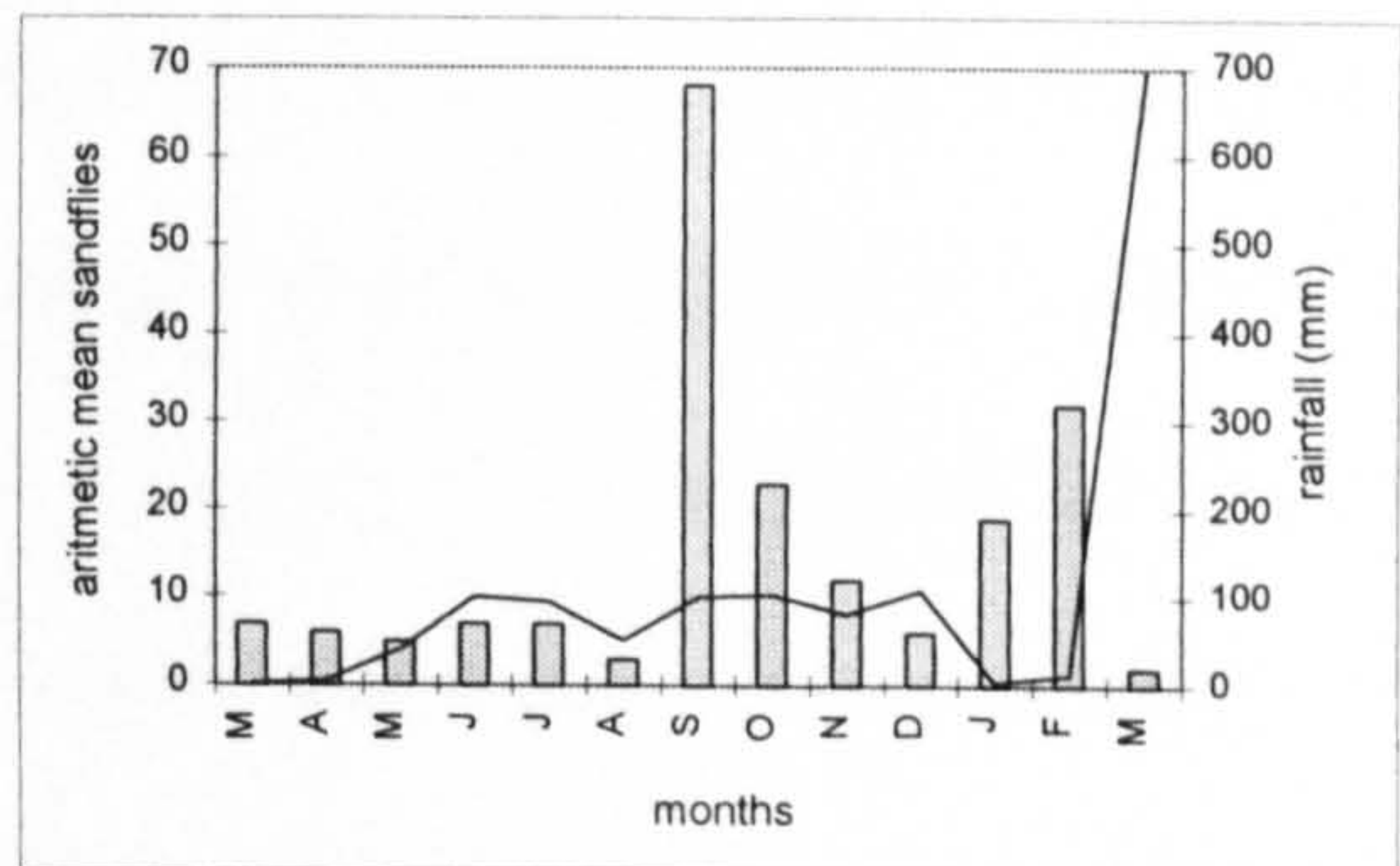
c) Limbo, Panama (Chaniotis et al, 1971)



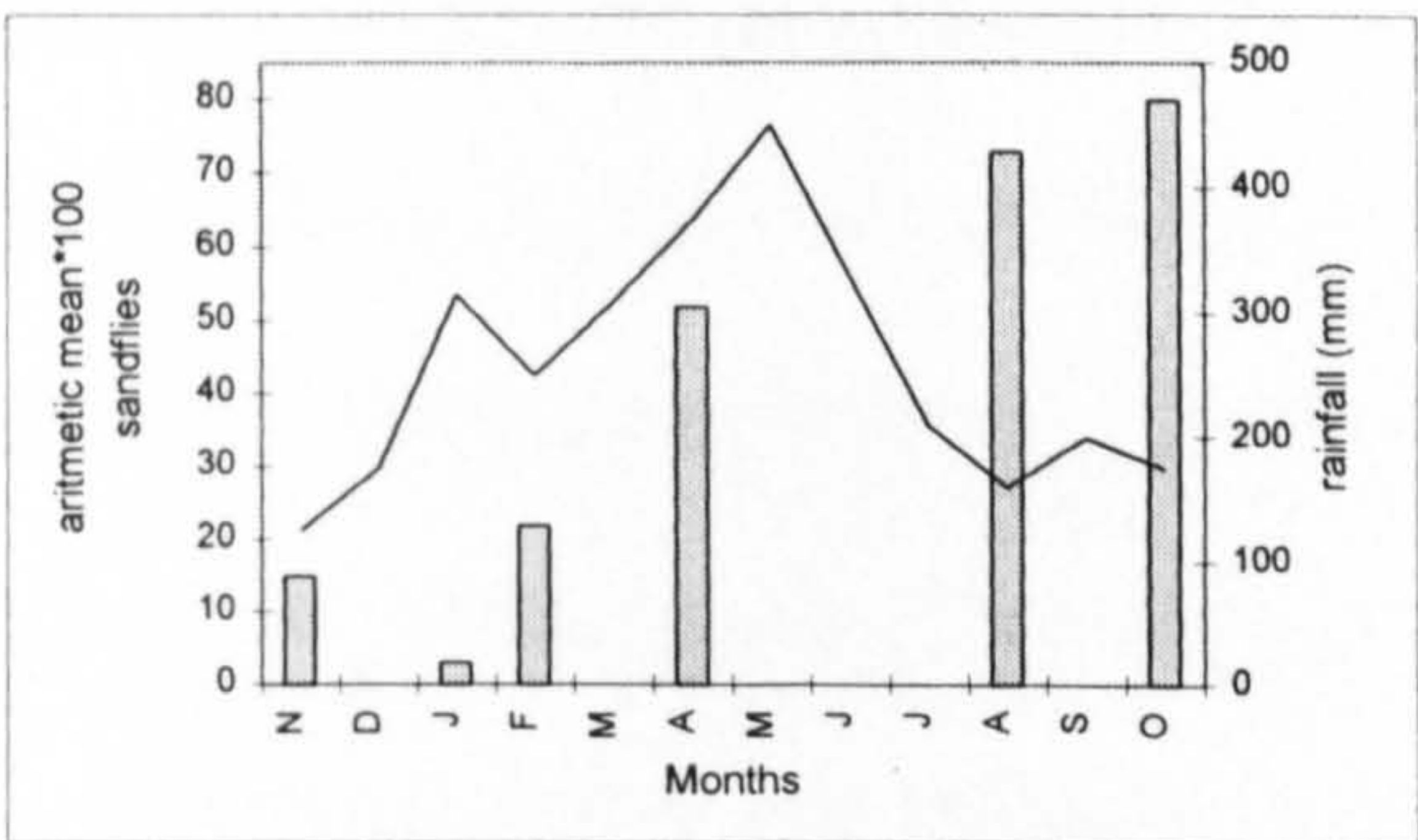
b) Paraiso Escondido, Ecuador (Le Pont et al, 1994)



d) San Estaban, Venezuela (Feliciangeli, 1987)

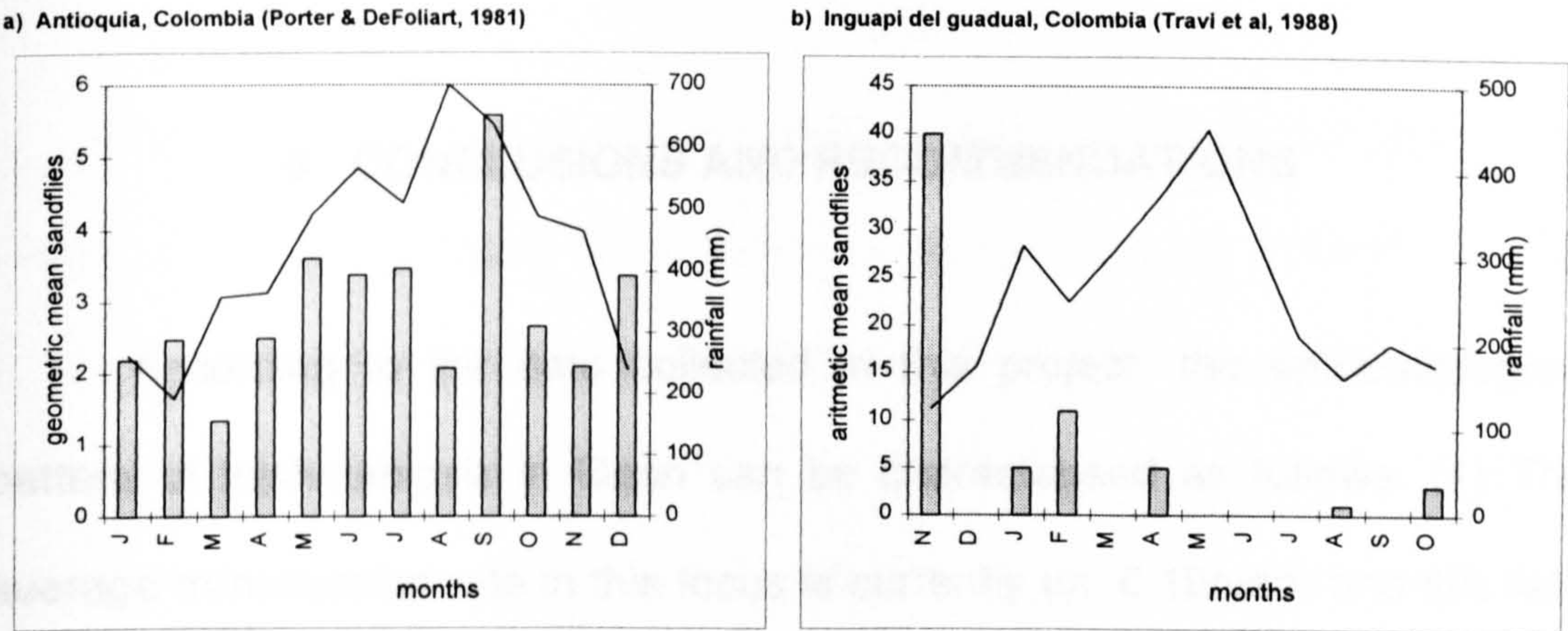


e) Inguapi del guadual, Colombia (Travi et al, 1988)



Bars represents sandflies abundance and solid line the rainfall patterns

Figure 5.21 Seasonal variation studies of *Lu. hartmanni* previously reported in Cental and South America



6. CONCLUSIONS AND RECOMMENDATIONS

According to the data collected in this project, the epidemiological pattern of leishmaniasis in Opon can be characterised as follows: (1) The average transmission rate in this focus is currently ca. 0.19/year and this rate has remained relatively stable for many years. (2) The risk of infection is equal for both genders and for all ages. (3) The main vectors are *Lu. trapidoi* and *Lu. gomezi*, although a minor vectorial role for *Lu. quasitownsendi* remains possible; and a significant proportion of transmission to humans takes place indoors at night. (4) There is relatively weak seasonal variation in transmission, but there is some suggestion of a peak associated with the annual period of less frequent rainfall (i.e. the last three months of the year). (5) The widespread deforestation that characterises the Opon focus has not caused any reduction in the incidence of leishmaniasis, presumably because the sandfly vectors continue to breed successfully in the cacao plantations that have replaced much of the primary forest. However, the risk of infection appears to be less where primary forest has been replaced by pasture or secondary forest. There is some evidence to suggest that the changing

patterns of land use in Opon have favoured *Lu. gomezi* more than *Lu. trapidoi*.

According to the data collected in this project, the biological and clinical nature of leishmaniasis in Opon can be characterised as follows: (1) The principal agent is *L. panamensis*, although a minor role for *L. braziliensis* or *L. colombiensis* cannot be discounted. (2) The most typical clinical symptoms are cutaneous lesions. The average number of lesions in patients was two, and the maximum number was 13. The average lesion size was ca. 1.5 cm², again with a maximum size of 95 cm²; and lesions tended to be larger if patients were infected at a younger age. The site of lesions on the body also varied with age and gender: children were relatively more likely to have lesions on the face, women on the legs, and men on the torso. (3) Patients with cutaneous lesions also had a significant risk (ca. 10%) of developing mucosal leishmaniasis, which was characterised in Opon by low severity (small nasal ulcers or perforated septa only). (4) About 31% of all infections were subclinical, and this rate increased with age. (5) Infections, whether subclinical or clinical, led to considerable acquired protective immunity (ca. 82%), although the rate of secondary infections (presumably reactivations) was relatively high (ca. 10%/year) during the first two years following a primary clinical infection.

Potential interventions for reducing the burden of leishmaniasis in the Opon focus are suggested by these results, and their impact should be tested in future trials. These include: (1) the use of insecticide impregnated bed-nets to reduce the observed late night indoor biting rate of *Lu. trapidoi* (the principal vector) in particular; (2) the improvement of house conditions and the use of house barriers (such as insecticide impregnated netting in doors and windows, and the sealing of gaps between the planks which form the walls) so as to reduce the evening indoor biting rate of *Lu. gomezi*, especially; (3) health education, targeted at high risk groups, to encourage personal protection and avoidance of visits to high risk areas at the time when sandflies are more active, i.e. the peridomestic cacao plantations at dusk; and (4) the targeting of drugs for children when drug availability is insufficient for all cases.

The natural history of leishmaniasis caused by *L. panamensis* in Opon, as described in this study, can be extrapolated to other foci in the inter-Andean valleys where the patterns of land use after deforestation are similar to that in the Opon and where the racial characteristics of the people at risk are similar. In particular, a similar pattern of human-sandfly contact will be expected in areas of the Colombian Andes where cacao plantations are the main crop. However, it would be fruitful to carry out further entomological studies over a wider geographic range to determine the generality of the ecological association between cacao and both *Lu. trapidoi* and *Lu. gomezi*.

Clearly, one of the main unanswered questions about leishmaniasis in Opon concerns the animal reservoirs. A putative reservoir role for domestic dogs has previously been suggested for *L. panamensis*; and if this hypothesis is proven, it implies the possibility that *L. panamensis* could persist even in areas where deforestation (and hunting) has led to the local extermination of the sloth population. Hence, a full understanding of the distribution of *L. panamensis* in Colombia, and an ability to predict the likely effects of the continuing deforestation of the Andes, will only be possible when further studies are carried out on the putative reservoirs.

Other important findings from the project relate to the choice of appropriate strategies for primary health care. The simultaneous follow-up of treated patients from different villages is not usually possible because of the highly dispersed nature of rural communities typified by Opon. In Opon, this problem was solved by involving the community in the search, diagnosis, and treatment of cases. The establishment of such community networks should result in multiple benefits, both for the community and for the NLCP: (1) patients are treated by trained people; (2) the Glucantime® is stored in safe conditions; (3) the doses and the frequency of treatment are given following medical recommendations; (4) the reporting of side effects is more effective; and (5) when the course of treatment is interrupted by the patient, the drug

would remain available for future patients, hence avoiding the development of a black market.

Finally, it is hoped that the production of this thesis will provide the NLCP in Colombia with a “blueprint” methodology which should aid the cost reduction of future field studies designed to measure transmission rates and leishmaniasis risk factors. For example, incidence rates (force of infection) could be estimated from age prevalence curves calculated from cross sectional surveys (where transmission is stable and age-independent), instead of by following a cohort of susceptibles. Cohort studies are extremely expensive (i.e. ca. £20,000 in Opon), as large sample sizes are required when incidence rates are low. An estimate of incidence rate can then be used to indicate the approximate number of treatments needed in a particular focus for the following year. Similarly, risk factors for prevalence associated with vegetation type (for example) can be estimated from data collected in visits of short duration.

. The cost of the seasonal variation study described in this thesis was ca. £10,000. However, it is suggested that the key entomological features in each focus can be identified from a reduced number of collections made during a more reasonable number of visits. For example, in future studies of the sandfly vectors in the Colombian Andes, the measurement of sandfly abundance in different environments could be carried out in two collections in

each rainy season using an appropriated number of replicas. In Opon, vector incrimination by multiple regression analysis was shown to be a powerful tool for quantifying the relative roles of different species. For species, such as *Lu. trapidoi*, from which parasites have been isolated and characterised on frequent occasions and from widespread endemic zones, there seems little point in carrying out further searches for natural infections (even with novel technology), because this methodology is expensive, time consuming, and has a low probability of success. However, circumstantial incriminatory evidence from statistical associations will never be sufficient to prove a vectorial role for those species from whom parasites have never been isolated in the field. Hence, the statistical epidemiology approach will not replace the biological approach to vector incrimination for sandflies in Colombia, until all the suspected vectors have been sufficiently examined (i.e. field infections have been sought from large numbers and from many endemic sites).

In most Central American and Andean countries the implementation of first line research laboratories in vector biology, ecology, immunology or microbiology has been postponed, because the priority for current research has been to solve basic questions concerning the natural history of the more common tropical diseases in each endemic zone. In the case of leishmaniasis, fundamental research is also limited by the necessary diversion of research funds for the acquisition of drugs, which are imported

by the endemic countries at very high prices. Future research projects on leishmaniasis in Colombia will need to be focused on priority topics, chosen for their relevance to public health policy; and the design of these projects should be determined by the need to maximise their cost-effectiveness. This can probably best be achieved by collaborations between the different groups active in leishmaniasis research in Colombia, so as (1) to gain the benefit of sharing resources and expertise and (2) to develop a rational strategy for a medium- or long-term research programme on leishmaniasis throughout the country.

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APPENDIX 1

FICHA DE CENSO LEISHMANIASIS: OPON-SANTANDER-COLOMBIA

FECHA : 15-02-95

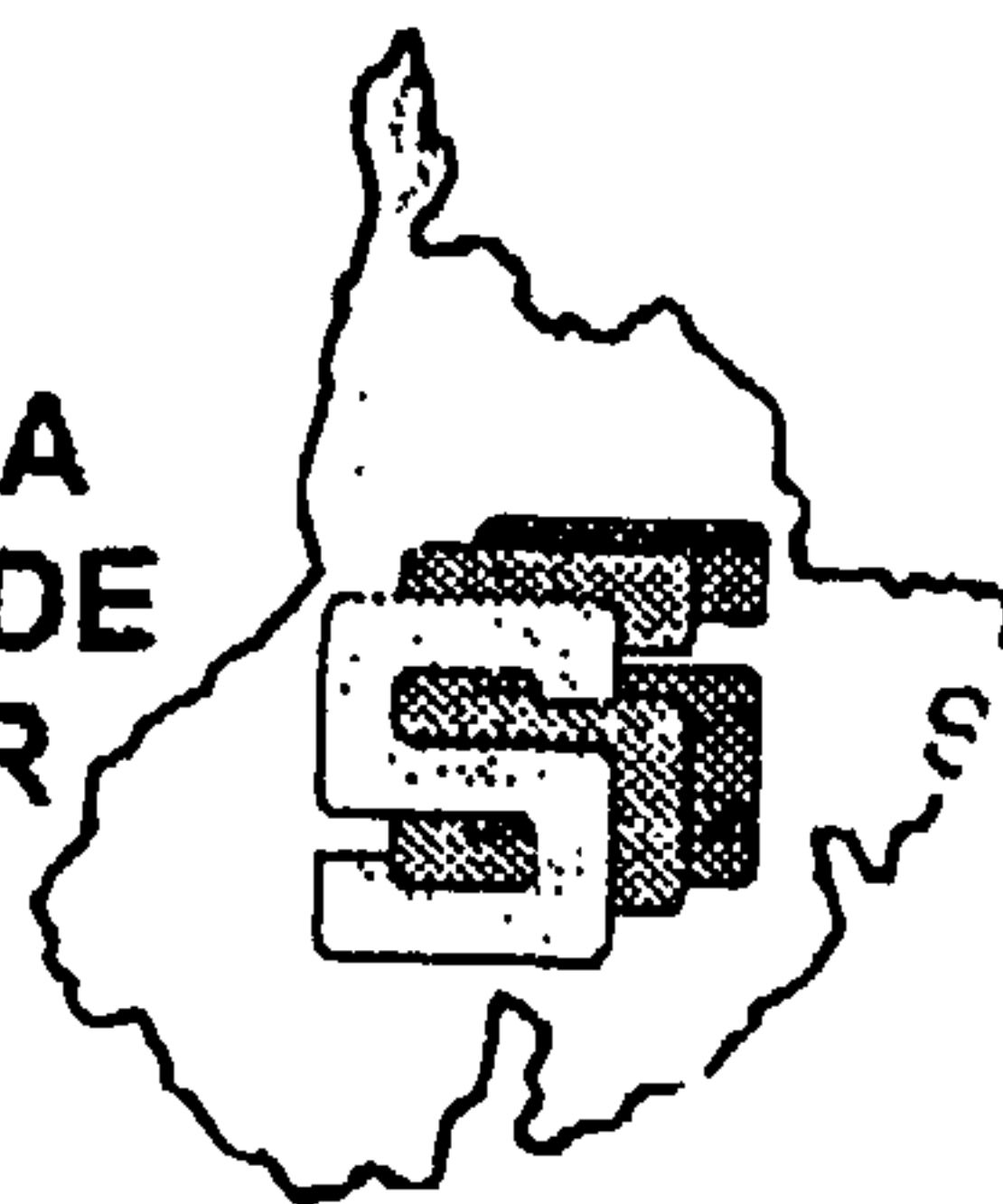
Nombre de la Vereda Plan de Atmas Codigo de la Casa: PA18 Altitud: 550 M

CODIG	NOMBRES Y APELLIDOS		Fecha/Nacim.	Sexo	ESTATUS		LEISHMANINA		INSPECCION		CAMBIOS EN POBLACION	
					INICIAL		FECHA	AREA	Numero	FECHA	ESTATUS	TIPO
PA1802	MAPIALUISA	PARDO	28-10-65	0	3		02-07-95	72	3	11-02-96		4
PA1801	PASION	DIAZ FLOREZ	19-04-62	1	4		02-07-95	484	2	11-02-96		4
PA1803	EDWARD	DIAZ PARDO	31-07-84	1	4		02-07-95	50	2	11-02-96		4
PA1804	WILMER	DIAZ PARDO	06-11-85	1	4		02-07-95	110	2	11-02-96		4
PA1805	SIDEY	DIAZ PARDO	22-08-88	0	1		02-07-95	0	1	01-07-95		1
PA1806	EMILIO	DIAZ PARDO	28-05-90	1	1		02-07-95	0	1	01-07-95		1
PA1807	FERNEY	DIAZ PARDO	22-10-92	1	1		02-07-95	0	1	01-07-95		1

**CINTROP
UNIVERSIDAD INDUSTRIAL
DE SANTANDER**



**SECRETARIA
DE SALUD DE
SANTANDER**



Fecha / /
DIA MES AÑO

1. Se aplico leishmanina : SI ☐ NO ☐

2. No se aplicó porque : Inmigrante: ☐ Es de otra vereda ☐ Embarazada ☐
Menor de 6 meses ☐ Renuente ☐

3. Menor de 10 años SI ☐ NO ☐

4. Nombre del paciente : _____

5. fecha de Nacimiento : ____/____/____ Edad : ____ años ____ meses Sexo: F ☐ M ☐

DIA MES AÑO

6. Nombre del acudiente : _____

7. Vereda _____ Código casa _____ Código paciente _____
8. Peso del paciente : _____ Kg.

2. **EFERMEDAD CUTANEA ACTUAL :** SI ☐ NO ☐

[illegible]

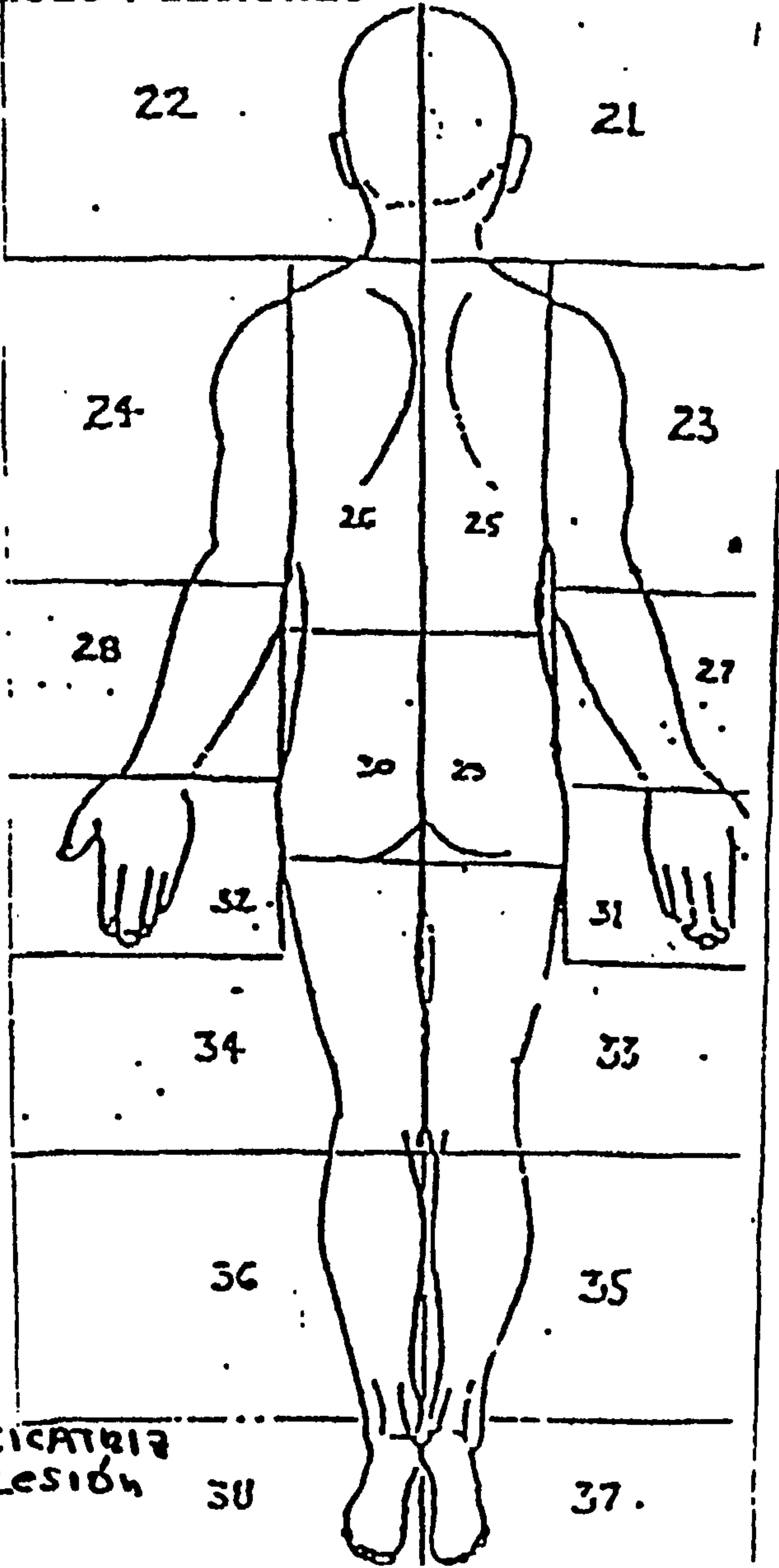
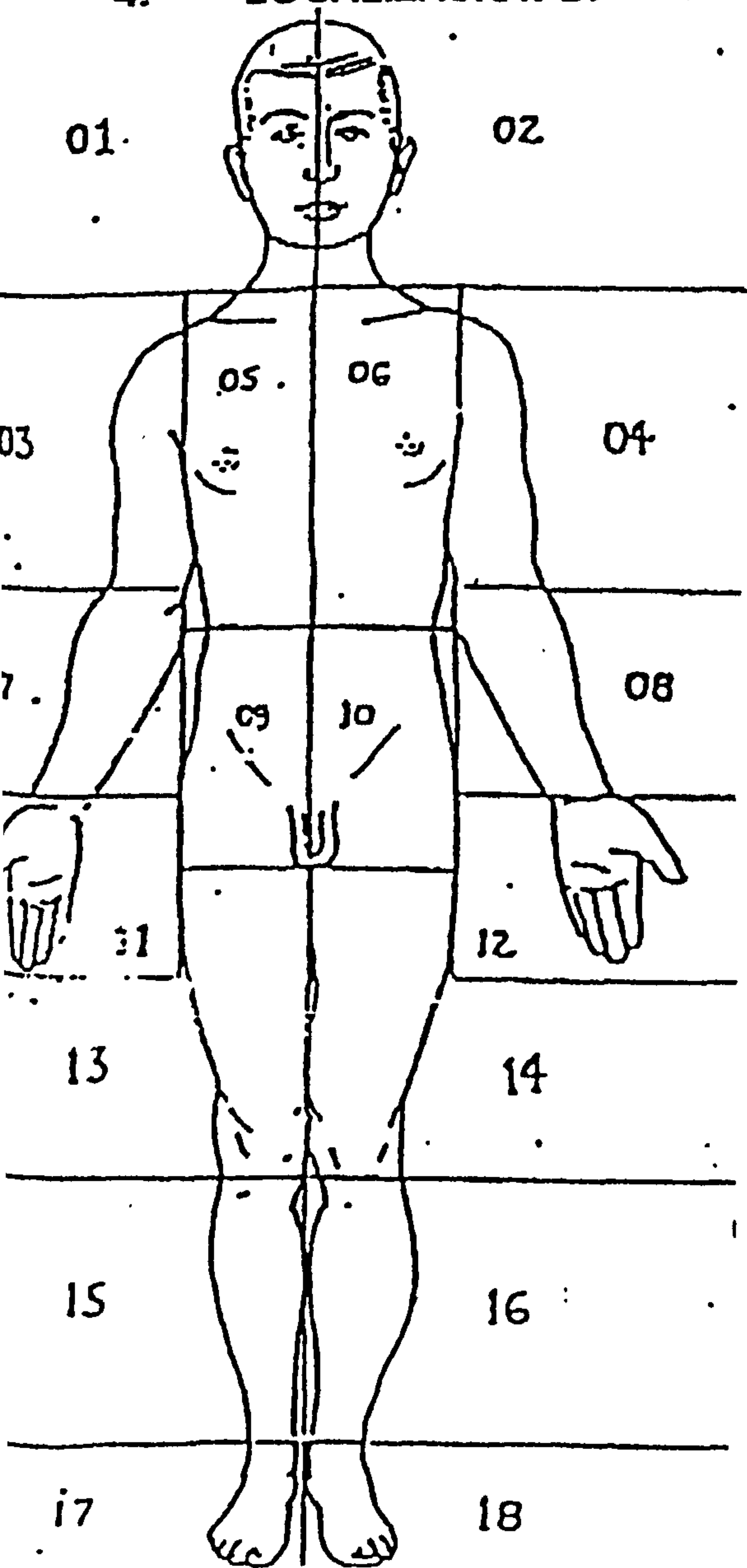
Infección secundaria presente : SI ☐ NO ☐ Cambios tróficos alrededor : SI ☐ NO ☐
adenopatías : SI ☐ NO ☐ Donde _____
Trayecto linfático inflamado : SI ☐ NO ☐ Cual _____

3. CICATRICES PRESENTES

código del paciente _____

NUMERO de la CICATRIZ	DIAMETRO	TIEMPO DE EVOLUCION	A QUE EDAD TUVO LA LESION

4. LOCALIZACION DE LAS CICATRICES Y LESIONES



X = CICATRIZ
O = LESION

5. ENFERMEDAD MUCOSA ACTUAL : SI ☐ NO ☐ código del paciente_____

Hiperhemia ☐ . Ulceración ☐. Infiltración ☐. Perforación ☐
Invasión a otros tejidos: SI ☐ NO ☐ Cuales tejidos:_____
Tiempo de evolución ____ Meses ____ Años Asintomático ☐

6. TRATAMIENTOS RECIBIDOS

Ha recibido Glucantime SI ☐ NO ☐
El tratamiento lo recibió por : La lesión actual ☐
Durante pasadas lesiones (actuales cicatrices) : ☐

Hace cuanto recibió la ultima ampolla : ____ días ____ meses ____ años ____ no recuerda
de ampollas aplicadas ____ Dosis ____ duración tratamiento ____ días
Vía administración : intramuscular ☐. intralesional ☐
Tipo de tratamiento : Empírico ☐. Médico ☐

7. DIAGNOSTICO

#	TIPO DE EXAMEN	1ros EXAMENES			RESULT	RESPONSA	2dos EXAMENES			RESULT	RESPONSABLE
		D	M	A			D	M	A		
	DIRECTO										
	CULTIVO										
	MONTENE										
	BIOPSIA										
	HAMSTER										
	PCR										

Si quedan exámenes pendientes escriba el #_____

8. ANTECEDENTES PERSONALES

TIPO CLASIFICACION	HEPATICAS		CARDIACOS		RENALES	
	SI	NO	SI	NO	SI	NO
LE VE						
MODERADO						
SEVERA						

